ISFG 2015

Krakow, August 31 – September 5, 2015

26th

Congress of the International Society for Forensic Genetics

26th Congress of the International Society for Forensic Genetics

Krakow, Poland
August 31 – September 5, 2015

ISBN:978-83-942760-1-0

26th Congress of the International Society for Forensic Genetics

Dear Friends and Colleagues,

We are very pleased that the 26th Congress of the International Society for Forensic Genetics is being held in one of the most beautiful and historic towns in Poland with a long and distinguished academic tradition – Krakow!

The conference program encompasses 10 workshops, during which recognised specialists will share knowledge in their fields with us, and plenary lectures, which will be given by eminent scientists with an indisputable influence on contemporary forensic genetics. The exceptionally rich scientific program, which includes 56 oral presentations and as many as 408 posters, will be additionally enhanced by an equally extensive exhibition program.

We believe that the 26th ISFG Congress offers great opportunities to present new research results, exchange ideas and opinions, draw inspiration and make scientific contacts.

For six days, the Auditorium Maximum of the Jagiellonian University will be transformed into a discussion forum for forensic geneticists from around the world. The conference venue is located just a few minutes from the historical centre of town, where there are numerous cafes, restaurants and clubs. We are confident that the unique atmosphere of Krakow and the exciting social program will help you to fully enjoy the conference.

The Congress would not be possible without the support of many people involved in its organisation, as well as its sponsors. We wish to extend our sincere gratitude to everyone who participated in the preparation of the 26th ISFG Congress.

Welcome to Krakow! We wish you a pleasant stay and look forward to a successful and intriguing congress.

Enjoy the meeting!

Tomasz Kupiec
Congress President

Wojciech Branicki
Congress Vice-President

TABLE OF CONTENTS

ABOUT ISFG

The International Society for Forensic Genetics is an international association promoting scientific knowledge in the field of genetic markers analyzed for forensic purposes. The ISFG has been founded in 1968 and represents more than 1100 members from over 60 countries. Regular meetings are held at a regional and international level. Scientific recommendations on relevant forensic genetic issues are developed and published by expert commissions of the ISFG. The international Congress of the ISFG is held every two years in various countries around the world.

LOCAL 2015 CONGRESS ORGANISING COMMITTEE

Tomasz Kupiec, Krakow
Wojciech Branicki, Krakow
Anna Tylek, Krakow
Maria Wróbel, Krakow

LOCAL 2015 CONGRESS OFFICE

Jagiellonian University Department of Communications and Marketing – Conferences

ISFG BOARD AND SCIENTIFIC COMMITTEE

Mechthild Prinz, New York
Leonor Gusmao, Porto
Niels Morling, Copenhagen
Walther Parson, Innsbruck
Peter M. Schneider, Cologne

CONGRESS VENUE

Auditorium Maximum of the Jagiellonian University, 33 Krupnicza St., Krakow

HONORARY PATRONAGE

MINISTER SPRAWIEDLIWOŚCI

Borys Budka
Ministry of Justice, Republic of Poland

KRAKÓW krakow.pl

Jacek Majchrowski
President of the City of Krakow

JAGIELLONIAN UNIVERSITY IN KRAKOW

Professor Wojciech Nowak
Rector of the Jagiellonian University

SPONSORS

The Organising Committee for the 26th Congress of the International Society for Forensic Genetics would sincerely like to thank for the sponsorship provided. Thanks to generous sponsorship, we were able to provide a platform for presenting the results of research as well as for heated discussion and searching for new ideas in forensic genetics.

GOLD SPONSOR

ThermoFisher SCIENTIFIC

Applied Biosystems™ products harness the power of science to transform lives. A member of the Thermo Fisher Scientific family of brands, our instruments, everyday tools, and services offer high-quality, innovative life science solutions for every lab and application. As a worldwide leader in forensics, we deliver some of the most comprehensively validated products, expertise, and application support available to the human identification community. From our trusted reagents, through high-performance genetic analyzers and software, to in-depth training and on-site technical assistance, our integrated systems work together to help maximize your productivity and enable your success. For more information, go to lifetechnologies.com/hid.

SILVER SPONSORS

GE Healthcare's Life Sciences business provides a wide variety of Whatman™ FTA™ products to meet the requirements of sample collection, transportation, and storage of DNA. FTA cards provide a simple solution to collect, preserve, and purify biological samples at room temperature for downstream DNA analysis. GE addresses the needs of its databasing and forensic customers with its Whatman FTA technology.

Illumina provides the most advanced systems for genetic analysis, including sequencing, genotyping, gene expression, and epigenetics. Our portfolio of tools is enabling advances in forensic genomics including targeted Next Generation Sequencing of forensically relevant loci, improved resolution of complex mixtures, and the ability to generate investigative leads.

Qualitype GmbH has over one decade of experience in the area of forensics and software development. As a bioinformatics company we develop software concepts contributing safety and efficiency in data management in forensic examination. With state-of-the-art technology and excellent trained staff Qualitype meets the manifold demands and challenges in biotechnology and bioinformatics. Qualitype GmbH is experienced in the realization of projects of various scale and complexity for many years. Besides standardized software products, our services range from innovative concepts to the development of effi cient solutions and their successful implementation. Additionally we also offer consulting, training and support for all our business areas.

WORKSHOP SPONSORS

ThermoFisher SCIENTIFIC

illumina®

NOTEPAD SPONSOR

integenX®

CONGRESS INFORMATION

CONGRESS VENUE

The conference will be held in the Auditorium Maximum of the Jagiellonian University, 33 Krupnicza St., close to the historical city centre. In 2001 the Senate of the Jagiellonian University decided to build a lecture theatre complex, Auditorium Maximum, located on Krupnicza Street. The investment plan proposed to build a complex of large modern lecture theatres in the immediate vicinity of Collegium Paderevianum. It includes the main theatre for 1200 persons, which can be divided into two smaller ones for 600 persons each if neccessary; one lecture room for 250 persons, two for 150 persons, and one for 100 persons, as well as the necessary catering and sanitary facilities.

The Auditorium Maximum facility was officially opened on September 9, 2005.

REGISTRATION

The reception desk is located on the ground floor next to the stairs in the Congress venue.

operating hours	August 31	Monday	08:30 – 18:00
	September 1	Tuesday	08:30 – 19:00
	September 2	Wednesday	08:30 – 18:00
	September 3	Thursday	08:30 – 18:00
	September 4	Friday	08:30 – 17:00
	September 5	Saturday	08:30 – 12:00

NAME BADGES

Delegates are kindly asked to pick their name badge at the Reception Desk. This badge is an individual official pass for entry to all sessions, lunches, the exhibition and social functions.

colour codes	
delegate	
invited speaker / workshop presenter	
organising committee / ISFG Board	
volunteer	
exhibitor	

ENTITLEMENTS

Congress registration fee includes: Admission to the scientific sessions (September 2 - September 5) and exhibitions, opening ceremony, congress materials, Get-Together Party, coffee breaks, lunches (September 2 - September 4), Congress Dinner. The Gala Dinner and Precongress Educational Workshops are not included in the congress fee.

Accompanying person fee includes: Get-Together Party, Congress Dinner, lunches (September 2 - September 4) and coffee breaks. Accompanying person fee does not include admissions to scientific sessions and congress materials. The Gala Dinner and Precongress Educational Workshops are not included in the congress fee.

CLOAKROOM

Cloakroom is located on the right side of the ground floor in the Congress venue.

SPEAKER PREPARATION DESK

Uploading speakers' presentations is proceeded at the Speakers Preparation Desk which is located next to the Reception Desk. All speakers are asked to provide their presentations 3-4 hours before their session (the day before for the first session in day).

COMPUTER SPOT & WIRELESS FACILITIES

All delegates are welcome to use computers located on the first floor of the Congress venue.

During the Congress free Wi-Fi in the venue will be available for the delegates.

login: **maximum**
password: **isfg2015**
Official hashtag of the event is: **#ISFG2015**

MESSAGES

All updates and important information will be available to read at the Reception Desk.

PHOTOGRAPHY

A professional photographer is hired for duration of the Congress. The pictures may be used for reports, case studies, marketing and supplied to the media if requested. If you do not agree to be photographed please notify the Reception Desk.

MOBILE PHONES

Please ensure that your mobile phones are switched off during Congress sessions.

SMOKING POLICY

The Congress venue is a non-smoking area. Delegates are requested not to smoke within the building

ISFG WORKING GROUPS MEETINGS

	German Group	Wednesday	18:00 – 19:00	Medium Hall A
	English Group	Wednesday	18:00 – 19:00	Large Hall
	French Group	Wednesday	18:00 – 19:00	Small Hall
	Italian Group	Wednesday	18:00 – 19:00	Medium Hall B
	Chinese Group	Wednesday	18:00 – 19:00	Conference Room
	Spanish/Portuguese Group (GHEP meeting)	Monday	09:00 – 18:00	Large Hall
		Tuesday	09:00 – 13:00	Large Hall

GENERAL ASSEMBLY OF THE ISFG

	For ISFG Members only	Thursday	18:00 – 19:00	Large Hall

COFFEE AND LUNCH BREAKS

Coffee and Lunch will be served in the Catering and Exhibition Area located on the -1 level. Additional Coffee stands will be on the second floor's hall of the Congress venue. Please remember to have your name badge with you.

	Monday 31.08.2015	Tuesday 01.09.2015	Wednesday 02.09.2015	Thursday 03.09.2015	Friday 04.09.2015	Saturday 05.09.2015
morning coffee	11:00 - 11:30	11:00 - 11:30	11:00 - 11:30	10:30 - 11:30	10:30 - 11:30	10:30 - 10:45 11: 30 -12:00
lunch	13:00 - 14:30	13:00 - 14:30	13:00 - 14:30	13:00 - 14:30	13:00 - 14:30	
afternoon coffee	16:00 - 16:30	16:00 - 16:30	16:00 - 17:00	16:00 - 17:00	16:00 - 16:30	

INCLUDED SOCIAL PROGRAM

	Concert: Marcin Wyrostek & Tango Corazon	September 1, 2015	20:15	Large Hall

Marcin Wyrostek - accordionist (born 16 September 1981); winner of the second edition of Poland's Got Talent; academic teacher at the Karol Szymanowski Academy of Music in Katowice; member of the American Accordionists' Association.

He is a laureate of over 30 accordion contests (in Poland and abroad), including Detroit (USA) 2005, Esztergom (Hungary) 2003, Dunajska Streda (Slovakia) 2003, Reinach (Switzerland) 2003. In the academic year 2004/2005, he was awarded the scholarship by the Minister of Culture and National Heritage.

He is the founder of two projects: TANGO CORAZON and COLORIAGE. He released three original records. One of the most exciting musical challenges for Marcin was performing together with Bobby McFerrin in 2010. His repertoire includes classical, jazz and

ethno-folk music. The key element of his musical identity is improvisation.

	Get-Together Party	September 1, 2015	Congress Venue -1 level	20:45 – 22:00
	Congress Dinner	September 4, 2015	Zalesie Manor Complex	20:00 – 00:00 *(bus departure: 19:00)*

The congress dinner will take place on September 4, 2015 in the Zalesie Manor Complex surrounded by forests, fields and meadows where you can enjoy a breathtaking view of the Beskidy and the Tatra mountains. The complex is situated 25 kilometers from Krakow.
Transportation service (buses) will be arranged for the congress participants.
Dress code: casual

OPTIONAL SOCIAL PROGRAMME

	Gala Dinner	September 2, 2015	Gallery of the 19th Century Polish Art in Sukiennice	20:00 – 23:00

The Gala Dinner will take place on September 2, 2015 in the Gallery of the 19th Century Polish Art in Sukiennice (the Cloth Hall, http://mnk.pl/oddzial/galeria-sztuki-polskiej).
Dress code: semi - formal
Please note that this option was available on a 'first come first served' basis due to the limited number of participants that can take part in the Gala Dinner.

GUIDELINES FOR ORAL AND POSTER PRESENTATIONS

The ISFG Board has carefully reviewed a large number of high quality abstracts and made every effort to develop a highly interesting and diverse scientific program. All authors of accepted oral or poster abstract are kindly asked to follow the instructions below.

ORAL PRESENTATIONS

Pre-viewing presentation will be available at the Speaker Preparation Desk – located next to the Reception Desk on the Ground Floor of the Congress venue. All speakers are asked to provide their materials 3-4 hours before their session (the day before for the first session in day). You are kindly requested to provide your presentation as a Microsoft Power Point file, which will be copied to the PC of the Congress venue. Personal laptops for presentation will not be accepted. Please make sure to bring adapters (MAC/PC plug) if necessary. The time length for each presentation must follow the schedule published in the Congress Program.

POSTER PRESENTATIONS

Posters shall be displayed during the whole congress and should not be mailed in advance. The poster should be placed in the appropriate presentation room, on the stand marked with poster number. The maximum acceptable poster size is L180xW90. Posters will be attached with tape provided in the poster session rooms.

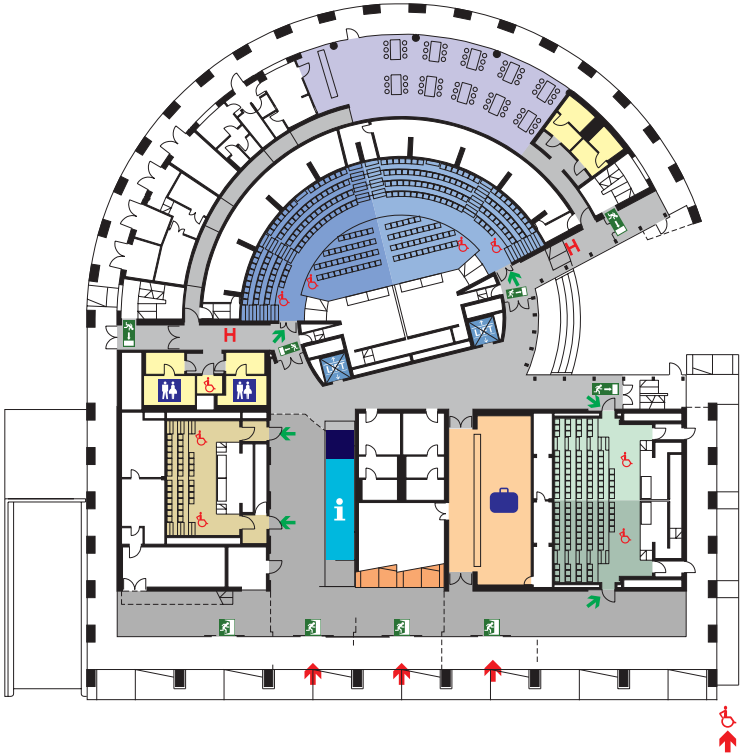
Posters can be set up from lunch time on Monday (August 31), and must be removed until Saturday (September 5) noon.

The presenting authors should be at their posters at the following times and place:

	Poster Theme	Poster Numbers	Day	Room	Time
1	Population genetics	1 – 30	Wednesday	2nd floor Exhibition Room – Poster Session	11:00 – 11:30
2	X - Chromosome variation	31 – 44			
3	Ethical and legal issues	45 – 50			
4	Lineage markers	51 – 117	Wednesday	2nd floor Exhibition Room – Poster Session	16:00 – 17: 00
5	Massively Parallel Sequencing	118 – 146			
6	New technologies	147 – 160			
7	Predictive DNA analysis	161 – 181	Thursday	2nd floor Exhibition Room – Poster Session	10:30 – 11:30
8	DNA typing methods	182 – 239			
9	DNA databases	240 – 246			

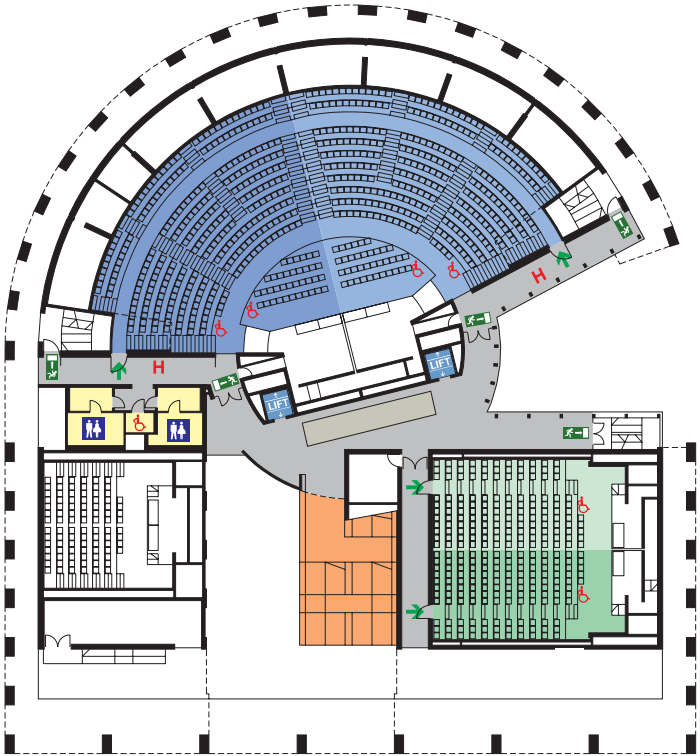
10	Body fluid identification	247 – 275	Thursday	2nd floor Exhibition Room – Poster Session	16:00 – 17: 00
11	Touch DNA	276 – 291			
12	DVI human remains	292 – 327	Thursday	2nd floor Seminar Room – Poster Session	
13	Biostatistics	328 – 349			
14	Paternity	350 – 363	Friday	2nd floor Seminar Room – Poster Session	10:30 – 11:30
15	Forensic Biology	364 – 374			
16	Legal medicine	375 – 386			
17	Non-human DNA	387 – 411			
18	Quality control	412 – 423			

PLANS OF AUDITORIUM MAXIMUM UJ



Ground Floor

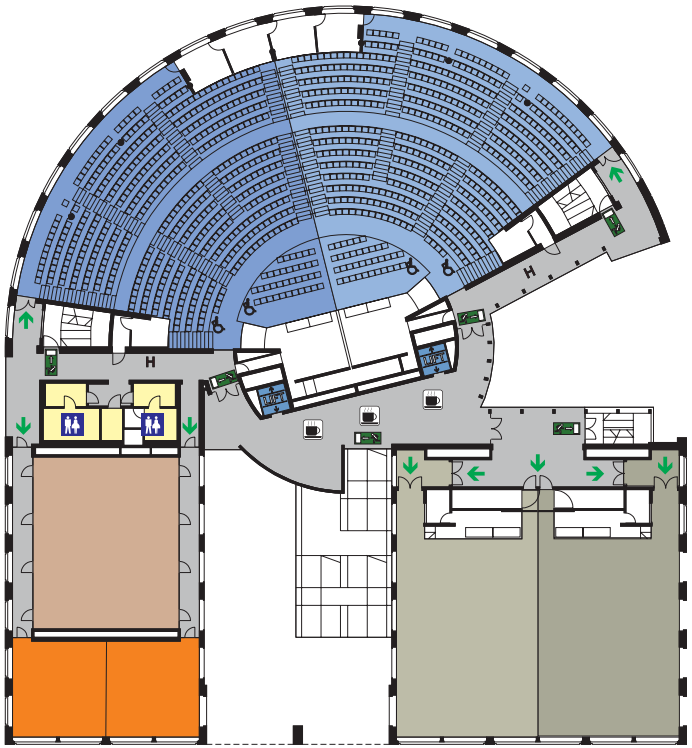
- Large Hall
- Medium Hall A
- Medium Hall B
- Small Hall
- Reception Desk
- Speaker Preparation Desk
- Entrance / Exit
- Entrance to Room
- Emergency Exit
- Lift
- Fireplug
- Cloakroom
- Toilets



First Floor

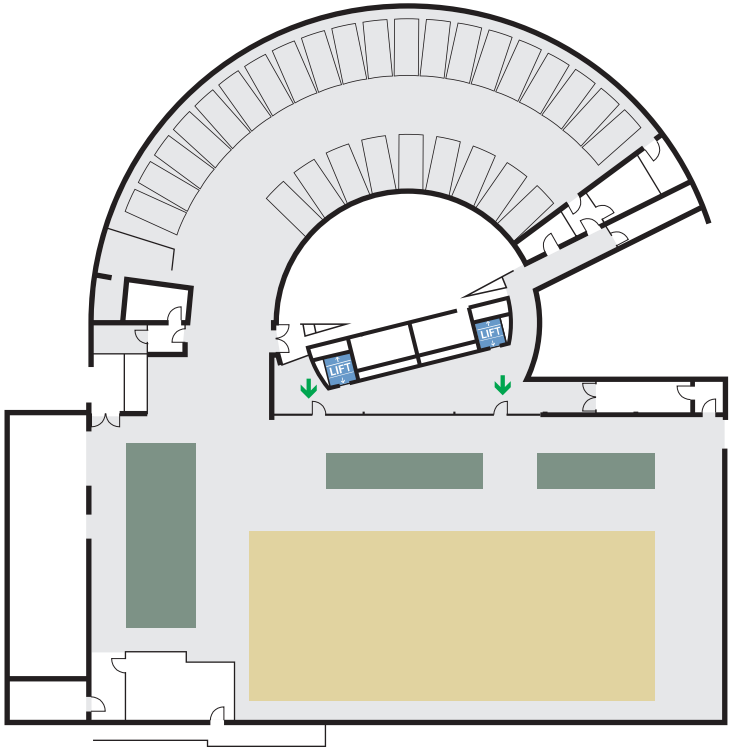
- Large Hall
- Medium Hall A
- Medium Hall B
- Computer Spot

- Entrance to Room
- Emergency Exit
- Lift
- Fireplug
- Toilets



Second Floor

- Large Hall
- Exhibition Room / Poster Session
- Conference Room
- Seminar Room / Poster Session
- Entrance to Room
- Emergency Exit
- Lift
- H Fireplug
- Toilets
- Coffee Area



-1 Level

Catering area

Exhibition area

Entrance

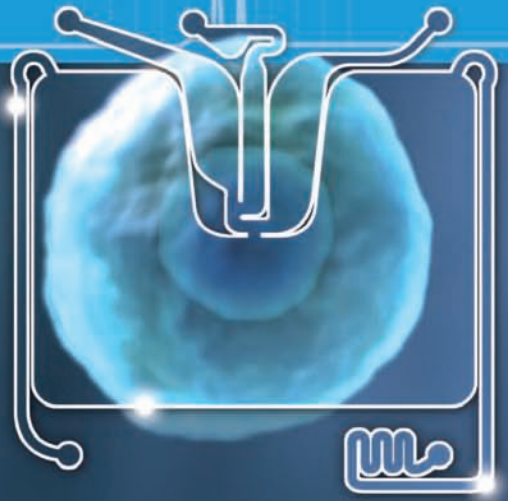
Lift

DEPArray™ TECHNOLOGY

PRECISE. FORENSIC. EVIDENCE.

DIGITALLY RESOLVING HETEROGENEITY IN BIOLOGICAL MIXTURES

The DEPArray™ system is a breakthrough technology that resolves heterogeneity in biological mixtures collected from unknown contributors. The precise isolation of 100% pure cells enables clear-cut genetic profiles from even small amounts of forensic biological evidences.



silicon biosystems

A MENARINI GROUP COMPANY

www.siliconbiosystems.com

KEYNOTE SPEAKERS

PETER GILL

Norwegian Institute of Public Health, Oslo, Norway
Department of Forensic Medicine, University of Oslo, Norway

I joined the Forensic Science Service (FSS) in 1982. I began research into DNA in 1985, collaborating with Sir Alec Jeffreys of Leicester University. In the same year we published the first demonstration of the forensic application of DNA profiling. In 1987 I was given an award under the civil service inventor's scheme for my discovery of the preferential sperm DNA extraction technique and the development of associated forensic tests. Currently I am Professor of Forensic Genetics, holding concurrent positions at the Norwegian Institute of Public Health and the University of Oslo.

In 1993-4 I was responsible for leading the team which confirmed the identity of the remains of the Romanov family, murdered in 1918, and also the subsequent investigation which disproved the claim of Anna Anderson to be the Duchess Anastasia (using tissue preserved in a paraffin wax block for several decades). This was one of the first examples of the solving of an historical mystery that involved the analysis of very degraded and aged material, and was one of the first demonstrations of low-template DNA analysis.

In relation to the above, I was responsible for developing a 'super-sensitive' method of DNA profiling that is capable of analysing DNA profiles from a handful of cells. This method was originally known as low-copy-number (LCN) DNA profiling (c.2000). Now it is known as Low template DNA profiling. New statistical methods and thinking were also developed to facilitate the new methods. In order to highlight the interpretation challenges, I recently published a book entitled: Misleading DNA Evidence: Reasons for Miscarriages of Justice (Elsevier).

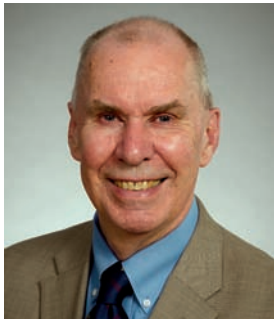
I was responsible for leading the team that developed the first STR multiplex DNA systems to be used to create a National DNA database and for the design of the matching/ interpretation methods that are still in current use (c.1995).

I am a member of the EU funded Euroforgen Network of Excellence, where I coordinate a work-package on the interpretation of evidence. I am actively promoting new methodology using open-source software to interpret complex DNA profiles by leading international workshops (including ISFG and CEPOL)

I have more than 200 publications with more than 14,000 citations.

BRUCE WEIR

Department of Biostatistics, University of Washington



Bruce Weir is Professor of Biostatistics at the University of Washington. He works in the general area of statistical genetics, most recently with whole-genome SNP and sequence data. He has an interest in forensic genetics: he coauthored an early textbook on DNA evidence interpretation with Ian Evett and he currently has a collaboration with John Buckleton and his New Zealand colleagues. He is a member of the Biology/DNA Science Advisory Committee established in 2014 by the US Department of Justice and the US National Institute of Standards and Technology. He is an Associate Editor of the Journal of Forensic Sciences.

CHRIS TYLER-SMITH

The Wellcome Trust Sanger Institute, Hinxton, Cambs. CB10 1SA, UK

I am head of the Human Evolution team at The Wellcome Trust Sanger Institute in Hinxton, UK. My background is in human molecular and evolutionary genetics, and from 1987-2003 I worked at the Department of Biochemistry, University of Oxford. In 2003, I moved to the Sanger Institute. My current work focuses on understanding the extent and distribution of genetic variation in human populations, related species such as gorillas, and exploring the evolutionary insights these provide. Most of this work involves whole-genome sequencing, both as part of international collaborations such as the 1000 Genomes Project, and as additional projects focusing on populations of particular interest.

MANEL ESTELLER

Institute for Biomedical Research (IDIBELL), Barcelona

Manel Esteller graduated in Medicine from the Universitat de Barcelona in 1992, where he also obtained his Ph.D. degree specialising in molecular genetics of endometrial carcinoma, in 1996. He was an Invited Researcher at the School of Biological and Medical Sciences at the University of St. Andrews, (Scotland, UK) during which time his research interests focused on the molecular genetics of inherited breast cancer. From 1997 to 2001, Esteller was a Postdoctoral Fellow and a Research Associate at the Johns Hopkins University and School of Medicine, (Baltimore, USA) where he studied DNA methylation and human cancer. His work was decisive in establishing promoter hypermethylation of tumour suppressor genes as a common hallmark of all human tumours. From October 2001 to September 2008 Manel Esteller was the Leader of the CNIO Cancer Epigenetics Laboratory, where his principal area of research were the alterations in DNA methylation, histone modifications and chromatin in human cancer. Since October 2008, Dr Esteller is the Director of the Cancer Epigenetics and Biology Program (PEBC) of the Bellvitge Institute for Biomedical Research (IDIBELL) in Barcelona, Leader of the Cancer Epigenetics Group, Professor of Genetics in the School of Medicine of the University of Barcelona, and an ICREA Research Professor. His current research is devoted to the establishment of the epigenome maps of normal and transformed cells, the study of the interactions between epigenetic modifications and non-coding RNAs, and the development of new epigenetic drugs for cancer therapy.

Author of more than four hundred fifty original peer-reviewed manuscripts in biomedical sciences, he is also a Member of numerous international scientific societies, Editorial Boards and reviewer for many journals and funding agencies. Dr Esteller is also Associate Editor for Cancer Research, The Lancet Oncology, Carcinogenesis and The Journal of The National Cancer Institute, Editor-in-Chief of Epigenetics and President of the Epigenetics Society.

ROBIN WILLIAMS

Faculty of Health & Life Sciences at Northumbria University

Robin Williams is Professor of Forensic Science Studies in the Faculty of Health & Life Sciences at Northumbria University, Professor Emeritus in the School of Applied Social Sciences at Durham University,and a Visiting Professor at the Policy, Ethics and Life Sciences Research Centre, Newcastle University. He was a member of the UK Nuffield Council of Bioethics Working Party on the Police Uses of Bioinformation and collaborated in writing their report published in 2008. More recently he worked (together with Carole McCartney and Tim Wilson) on the Nuffield Foundation project on 'The Future of Forensic Bioinformation'. His published work includes (with Paul Johnson) 'Genetic Policing: The Use of DNA in Criminal Investigations' (Willan, 2008) and (edited with Jim Fraser), 'The Handbook of Forensic Science' (Willan, 2010). His previous studies of the organisation of forensic science support to policing and the growth of forensic DNA databasing have been funded by the UK Home Office and the Wellcome Trust. As well as being the NUCFS (Northumbria University Centre for Forensic Science) lead investigator on the FP7 funded European Forensic Genetics Network of Excellence' (EUROFORGEN), he currently holds a Wellcome Trust grant to support a study of the use of 'familial searching' in serious crime investigations in the UK and the USA, and is a co-investigator on a 3 year Leverhulme Trust funded study of the use of forensic science to support homicide investigations in Great Britain: 'Homicide Investigation and Forensic Science: Tracing Processes, Analysing Practices'.

TOMASZ GRZYBOWSKI

Forensic Medicine Institute at the Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland

Professor Tomasz Grzybowski is the chairman of Forensic Medicine Institute at the Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland. His research interests include: population genetics, molecular phylogeography and phylogenetics, applied to the reconstruction of the prehistory of human populations. Over the last decade, he has performed many studies on the populations of Western and Eastern Eurasia, employing a variety of genetic markers (mitochondrial DNA, Y- STRs and SNPs and autosomal SNPs). Prof. Grzybowski has pioneered the forensic applications of complete mitogenome analyses in Poland. His population research into haploid markers has contributed to the deeper insight into the origin and initial expansion of Slavs in Europe.

ZERO MEANS ZERO WITH POWER QUANT

Confidence Comes from Clear Vision.

Promega

Promega

POWERPLEX® FUSION SYSTEMS

PENTA E SE33 PENTA D

DYS576 D10S1248 D3S1358 DYS570

DYS391 D1S1656 D2S1338

More Loci. More Choices. More Information.

The PowerPlex® Fusion Systems offer superior sensitivity, rapid cycling and direct-amplification capabilities. The new **PowerPlex® Fusion 6C System** combines those advantages with SE33 and two additional Y-STR loci for improved mixture deconvolution and unparalleled performance with challenging casework samples.

PERFORMANCE PACKED.

To learn more: **www.promega.com/FusionRocks**

WORKSHOP PROGRAM

BASIC STR INTERPRETATION

Date: August 31, 2015
Time: 9:00 – 17:00
Room: Medium Hall A

Workshop presenters: Butler J. M., Ph.D. (NIST) and Gittelson S. N., Ph.D. (NIST)

Objectives: To provide easy-to-follow, basic-to-intermediate level information and to introduce key concepts and fundamental literature in STR data and statistical interpretation.

EMPOP ADVANCED PRACTICAL COURSE

Date: August 31, 2015
Time: 9:00 – 18:30
Room: Conference Room

Workshop presenters: Xavier C., Bodner M., Parson W.

Aim: This workshop introduces into the new database EMPOP 3 and gives hands-on practice using casework examples.

Learning outcome: Participants are made familiar with the new functionality of EMPOP 3 and learn how to use the website for: database queries for frequency estimation, retrieving statistical information on common and rare haplotypes, forensic reporting of mtDNA data, searching and interpreting point and length heteroplasmy, estimating the haplogroup status of an mtDNA sequence, interpreting geographic distribution of haplotypes and haplogroups.

NEXT GENERATION SEQUENCING

Date: August 31, 2015
Time: 9:00 – 18:30
Room: Medium Hall B

Workshop presenters: Morling N., Børsting C., Irwin J., Schellberg T., Vallone P. M., Williams R., Brion M., Budowle B.

Agenda: introduction to multiple parallel sequencing (MPS, NGS, SGS), Forensic genetic MPS – overview, Forensic genetic MPS and data analysis options, Sequencing of SNPs and STRs with MPS for forensic genetic, Forensic genetic MPS – sequence diversity of STRs, Forensic genetic MPS of mtDNA, MPS and molecular genetics of sudden cardiac death in the forensic setting, Will NGS lead to significant expansion of the core loci? Identify benefits and policy/legal issues, Biolegal innovations and the public good: recent socio-ethical observations on criminal justice applications of Massively Parallel Sequencing.

Morning Session is sponsored by ThermoFisher Scientific. Afternoon session is sponsored by Illumina.

BEYOND DNA-PROFILING: RNA-PROFILING, TRANSFER AND PERSISTENCE – WHAT IS IT AND HOW DID IT GET THERE?

Date: August 31, 2015
Time: 9:00 – 18:30
Room: Small Hall

Workshop presenters: Harbison S. A., Ballantyne J., van Oorschot R. A. H., Sijen T.

Aim: This workshop describes and discusses methods and insights that stretch beyond determining who is the donor of an evidentiary stain. The first part of the workshop will focus on mRNA profiling that is used in casework to infer what cell type(s) are present. The second part will address recovery, transfer and persistence of human cell material and how such knowledge may assist the interpretation of forensic cases at the activity level.

Learning outcome: Participants are made familiar with

the ins and outs of mRNA profiling and how RNA results can be used in forensic cases in which also DNA results were obtained. In addition, the participants gain understanding of the prevalence of human cell material on various locations and how issues of recovery, transfer and persistence can affect how much and whose cell material is present. An excursion to interpretation at activity level will be made.

THE INTERPRETATION OF COMPLEX DNA PROFILES USING OPEN-SOURCE SOFTWARE LRMIX STUDIO AND EUROFORMIX (EFM)

Date: September 1, 2015
Time: 9:00 – 18:30
Room: Conference Room

Workshop presenters: Gill P., Benschop C., Hansson O., Bleka Ø.

The focus of the workshop will be to teach the principles of complex mixture interpretation facilitated by the two models. There is no gold standard model, hence the emphasis of the training will be with LRmix Studio as this remains our preferred method. However, the availability of EuroForMix model will enable users to carry out their own comparative studies between the two types of model that are currently discussed in the literature.

KINSHIP ANALYSIS

Date: September 1, 2015
Time: 9:00 – 13:00
Room: Small Hall

Workshop presenters: Egeland T., Slooten K.

Given DNA data and possibly additional information like age on a number of individuals, we may ask the question: "How are these people related"? The workshop presents methods to address this problem emphasizing statistics.

If genetic data are possibly uncertain due to allelic dropout or dropin, the traditional likelihood formulas no longer apply and probabilistic assessment of the data is necessary. We will discuss one such approach where a model using dropout and dropin is used (similarly to how this is done for mixture evaluation). We apply this approach to a number of scenarios, e.g. pairwise kinship testing and familial searching in a database. We furthermore show how cases can be pre-assessed by determining likelihood ratio distributions, including the case where there is uncertainty about the genetic data.

Learning outcome: Obtain an overview of recent developments for relationship inference involving replicated DNA profiles of poor quality.

THE NEW Y CHROMOSOME HAPLOTYPE REFERENCE DATABASE AND OPTIMIZED APPROACHES FOR THE FORENSIC Y-STR ANALYSIS

Date: September 1, 2015

Time: 14:30 – 18:30

Room: Small Hall

Workshop presenters: Roewer L., Willuweit S.

Aim: This workshop introduces into the new database YHRD version 4.0 and gives hands-on practice using casework examples. Participants learn to understand the dual workflow (parallel autosomal and Y-chromosomal STR analysis) applied for mixed traces in sexual assault casework.

Learning outcome: The participants will understand how a combination of autosomal and Y-STR analysis can increase the number of probative DNA profiles. The participants will learn how to use the YHRD website for: interpreting Y-chromosomal evidence, database queries for frequency estimation (observed and expected, constant and variable frequency estimators), LR based male/male mixture interpretation, LR based kinship calculation using a lineage marker (Y), calculation of population differentation indices (Fst, Rst), retrieval of ancestry information.

ETHICAL, LEGAL AND SOCIAL ISSUES IN FORENSIC GENETICS

Date: September 1, 2015

Time: 9:00 – 13:00

Room: Medium Hall A

Workshop presenter: Williams R., Wienroth M., Syndercombe Court D., Reed K.

Aims: The aims of the workshop are (i) to review the major recurrent social, ethical and legal issues that have arisen surrounding the introduction and development of forensic genetics in support of criminal investigations and prosecutions, and (ii) to encourage participants to consider how best to engage with these issues as forensic science stakeholders and practitioners.

Learning outcomes: By the end of the workshop participants will:

- understand the history of Ethical, Legal and Social Issues (ELSI) deliberations in forensic genetics and the relationship

of this history to wider social and ethical contexts and considerations;

- appreciate the variety of regulatory frameworks which exist to govern the uses of forensic genetics in support of criminal justice;
- be able to relate general ethical and legal frameworks to the use of particular forensic genetic technologies in practical case settings;
- have considered the difference between a willingness to follow ethico-legal prescriptions and the development of autonomous ethical reflexivity as a basis for professional practice;
- have resources that will enable them to engage in policy deliberations about the future nature and uses of forensic genetics.

FORENSIC DNA PHENOTYPING: BASIS, AVAILABILITIES AND EXPECTATIONS

Date: September 1, 2015
Time: 14:30 – 18:30
Room: Medium Hall A

Workshop presenters: Branicki W., Kayser M., Pośpiech E., Walsh S.

Aim of the workshop: This workshop provides education on the current state-of-the-art in the field of DNA prediction of human appearance traits (i.e., Forensic DNA Phenotyping, FDP).

Learning outcome: Participants of the workshop are provided with knowledge on: the theoretical basis of Forensic DNA Phenotyping: terms and definitions, identification and selection of prediction markers, prediction modeling and evaluation of prediction outcomes; overview on DNA-based prediction methods and tools currently available for pigmentation traits; hands-on advice on DNA predicting eye and hair colour using the HIrisPlex system; DNA prediction of scalp hair distribution in males; DNA prediction of human age using epigenetic markers; future perspectives on appearance DNA prediction.

INTERPRETATION OF COMPLEX DNA PROFILES USING A CONTINUOUS MODEL – AN INTRODUCTION TO STRMIX

Date: September 1, 2015
Time: 9:00-17:00
Room: Medium Hall B

Fee: This workshop is free to ISFG attendees, but spaces are limited

Workshop presenters: Bright J. A., McGovern C. E., Taylor D.

Workshop description: STRmix™ is expert forensic software that can resolve previously unresolvable mixed DNA profiles. It uses a fully continuous approach for DNA profile interpretation, resolving complex DNA mixtures with no restriction on the number of contributors. STRmix™ is the standard for DNA profile interpretation in Australia and New Zealand.

This workshop includes an introduction to probabilistic software including a hands-on demonstration of STRmix™. Theory topics include the principles of, advantages and difficulties of probabilistic genotyping, principles of using peak heights and stutter ratios and the motivation to change. Attendees will walk through the interpretation of a number of complex and mixed DNA profiles using STRmix™ as a class. The class will explore the variability in likelihood ratios, and the run diagnostics within STRmix™. In addition, there will be a discussion on the validation and implementation of STRmix™ within a laboratory.

COMPANY SYMPOSIA

The symposia listed below are optional and not included in any registration category for signing in please visit websites mentioned; there is no additional cost to attend.

THERMOFISHER SCIENTIFIC LUNCH SYMPOSIUM

ThermoFisher SCIENTIFIC

Title: Forensic genetics and massively parallel sequencing. Technology fundamentals, data analysis and implications for the future on forensic DNA analysis
Date: Wednesday, September 2, 2015
Time: 13:00 – 14:00
Room: Medium Hall A and B
Registration: lifetechnologies.com/isfg2015
Keynote Speaker: Prof. Dr. Bruce Budowle, Executive Director of Institute of Applied Genetics, Texas, US

PROMEGA CORPORATION LUNCH SYMPOSIUM

Promega

Date: Thursday, September 3, 2015
Time: 13:00 – 14:00
Room: Medium Hall A and B
Registration: http://www.promega.de/resources/events/deu/de_1505_isfg/
Direct registration: http://promega.formstack.com/forms/?2021501-mLbsCZmMFA
Keynote Speaker: Peter de Knijff, others TBD

QIAGEN LUNCH SYMPOSIUM

Title: QIAGEN's New Investigator Technologies Symposium
Date: Friday, September 4, 2015
Time: 13:00 – 14:00
Room: Medium Hall A and B
Registration: https: //www.qiagen.com/de/landing-pages/regional/europe/events/isfg2015?sc_mode=normal

SILICON BIOSYSTEMS PRESENTATION

Title: Digitally resolving mixed biological evidence with DEPArrayTM technology: 100% pure profiles from each contributor
Date: Thursday, September 3, 2015
Time: 10:30 – 11:00
Room: Medium Hall A and B
Registration: https://www.eventbrite.com/e/silicon-biosystems-symposium-isfg-2015-tickets-17715992009
Keynote Speaker: Dr. Raimo Tanzi (Chief Commercial Officier at Silicon Biosystems)
Dr.ssa Francesca Fontana (Biology R&D Manager at Silicon Biosystems)

EXHIBITIONS

The Trade Exhibition is located on the -1 level of the Auditorium Maximum building next to the Catering Area. All delegates are invited to visit the exhibition and meet with the company representatives during the Congress.

EXHIBITION OPERATING HOURS

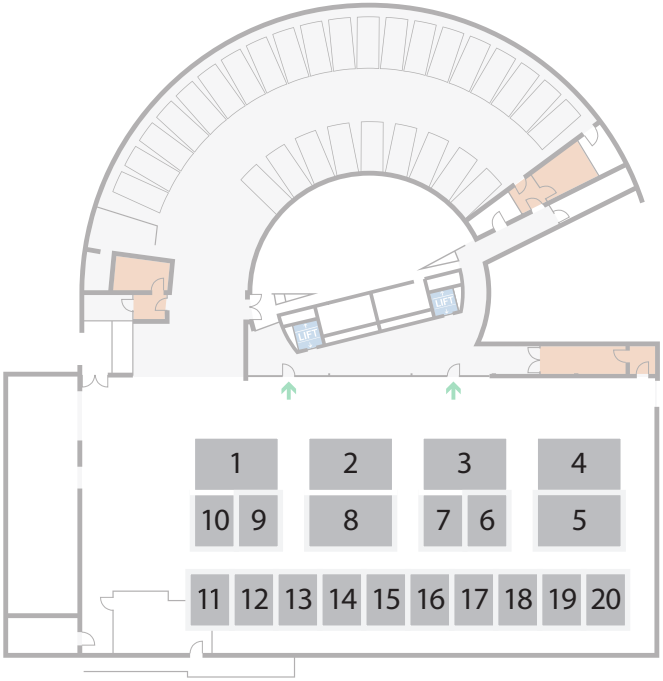
Monday, August 31	9:00 – 18:00
Tuesday, September 1	9:00 – 18:00
Wednesday, September 2	9:00 – 18:00
Thursday, September 3	9:00 – 18:00
Friday, September 4	9:00 – 18:00
Saturday, September 5	9:00 – 12:00

COMPANY BOOTH NUMBERS

1 Thermo Fisher Scientific
2 Illumina Inc.
3 STRmixTM / NicheVision Forensics
4 AQUALAB
5 HAMILTON Bonaduz AG
6 InnoGenomics Technologies
7 Silicon Biosystems
8 QIAGEN GmbH
9 Qualitype GmbH
10 GE Healthcare
11 Promega Corporation
12 IntegenX Inc.
13 Copan Flock Technologies
14 Health Gene Technologies Co.
15 SERATEC GmbH
16 Bode Cellmark FORENSICS
17 STACS DNA
18 ELSEVIER
19 ISFG 2017 Congress Host
20 LGC Standards

Exhibiting companies are listed in an alphabetical order.

EXHIBITORS PLAN



AQUA LAB

Booth number: **4**
Contact person: Anna Gozdalik
Telephone: 0048226769028 ext 103
E-mail: agozdalik@aqualab.pl
Website: www.aqualab.pl

Sales of laboratory equipment and chemical reagents. Manufacturing and servicing of various laboratory instruments. Training in the field of accreditation of laboratories. Exclusive representation on territory of Poland Macherey-Nagel GmbH & co. (since 1994) Hamilton Bonaduz AG (since 2010) .

Bode Cellmark FORENSICS
LabCorp Specialty Testing Group

Booth number: **16**
Contact person: Manzar Ahmed, Strategic Director – International Business

Telephone: M: +1 202-489-6302
O: +1 703-646-9805
E-mail: Manzar.Ahmed@bodetech.com
Website: www.bodetech.com

Bode Cellmark Forensics has served the legal and law enforcement communities for more than 25 years. Through a rich history of technical expertise and innovations in the field of forensic science, Bode Cellmark has a proven track record of assisting with the implementation of highly-efficient, cost-saving operational strategies. Bode Cellmark's laboratory personnel have analyzed more than 3.5 million samples, more than 140,000 forensic cases, and more than 30,000 unidentified human remains. Our scientists have provided expert witness testimony in more than 1,000 forensic cases. Decades of experience make Bode Cellmark one of the world's most trusted companies for individual and high-volume forensic DNA analysis services.

COPAN | COPAN FLOCK TECHNOLOGIES
Innovating Together™

Booth number: **13**
Contact person: Mr. Nicola Arrighi
Telephone: +39-3495715300
E-mail: nicola.arrighi@copanitalia.com
Website: www. copanflocktech.com

Copan Flock Technologies, a division of the COPAN Group, is dedicated to developing innovative devices for sample collection, transport, preservation and processing to serve diagnostic, biotechnology and life science companies including forensics. Copan Flock Technologies (CFT) is unique within the COPAN group in many respects. COPAN's flagship innovation, FLOQSwabs™, opened the door for CFT to further expand its capabilities to create improved devices, not just swabs, for collecting and eluting samples used in a wide variety of applications from molecular assays, antigen testing and EIA to Forensics, DNA paternity kits, and drugs of abuse testing kits. CFT is a total solution provider that works in partnership with industry clients to develop customized or unique products.

ELSEVIER

Booth number: **18**
Contact person: Ruslana Khatagova
Telephone: + 44 20 7424 4302
E-mail: r.khatgova@elsevier.com
Website: www.elsevier.com

Elsevier is a leading publisher of scientific, technical and medical information products and services. The company works in partnership with the global science and health communities to publish more than 2,000 journals. We are dedicated to delivering world-class content and superior information solutions in a variety of print and electronic formats.

Booth number: **10**
Contact person: Tom Naven
Telephone: +447799582034
E-mail: tom.naven@ge.com
Website: www.gelifesciences.com/forensics

GE Healthcare's Life Sciences business provides a wide variety of Whatman™ FTA™ products to meet the requirements of sample collection, transportation, and storage of DNA. FTA cards provide a simple solution to collect, preserve, and purify biological samples at room temperature for downstream DNA analysis. GE addresses the needs of its databasing and forensic customers with its Whatman FTA technology.

HAMILTON
THE MEASURE OF EXCELLENCE ™

Booth number: **5**
Contact person: Dr. Jörg Katzenberger
Telephone: +49 89 552 649 0
E-mail: infoservice@hamiltonrobotics.com
Website: www.hamiltonrobotics.com

Hamilton Robotics designs and manufactures fully automated robotic systems for sample preparation and storage. The products range from unique, custom laboratory automation solutions (turnkey solutions) on standard applications validated through partnership programs with renowned biotechnology companies as well as OEM solutions to the top ten diagnostic companies. Hamilton has proven automation solutions for the complete forensic workflow, from sample lysis, DNA extraction + quantificiation to normalization and STR/CE setup. The combination of great know-how of the engineers and experienced scientists in biotechnology,

drug discovery, diagnostics and software as well as the constant development and improvement allows Hamilton to satisfy customers' needs. Precision, innovation, reliability and quality are the philosophy of the technology leader Hamilton Robotics.

HEALTH
Gene Technologies

Booth number: **14**
Contact person: Ausma Bernotaite
Telephone: +86 183 5740 2013
E-mail: ausma.b@healthgenetech.com
Website: en.healthgenetech.com

Ningbo Health Gene Technologies Co., Ltd (HGT) dedicates to Forensic DNA applications. Equipped with 2000 m2 molecular diagnostic R&D laboratory and a 1000 m2 GMP standard reagent production plant, HGT offers four Forensic DNA profiling kits, which have completed more than 500,000 tests. Certified with TUV ISO9001:2008/ ISO13485:2012 and NQA ISO14001:2004, HGT carries out all its processes at the highest standard to define, develop, implement, validate, and transfer the pilot Forensic DNA Identification Kits production to volume manufacturing.

To learn more, visit www.healthgenetech.com

illumina®

Booth number: **2**
Contact person: Nicola Oldroyd, Market Development Manager, Forensic Genomics, Illumina
Telephone: +44 (0) 7585 702574
E-mail: noldroyd@illumina.com
Website: www.illumina.com/forensicgenomics

Illumina provides the most advanced systems for genetic analysis, including sequencing, genotyping, gene expression, and epigenetics. Our portfolio of tools is enabling advances in forensic genomics including targeted Next Generation Sequencing of forensically relevant loci, improved resolution of complex mixtures, and the ability to generate investigative leads.

InnoGenomics®
INNOVATION IS IN OUR GENES

Booth number: **6**
Contact person: Jonathan Tabak

Telephone: +1 (504) 459-4560
E-mail: jtabak@innogenomics.com
Website: www.innogenomics.com

InnoGenomics Technologies is dedicated to the development of innovative genetic testing solutions that solve crimes and save lives. Our patented technology enables forensic scientists to unlock answers from the most challenging DNA evidence. This includes the InnoTyper® 21 kit for recovery of interpretable, discriminating results from extremely degraded and/or low-level forensic samples, and the InnoQuant®/InnoQuant® HY kits for highly sensitive DNA quantification and degradation assessment. We also offer mock forensic samples simulating complex casework scenarios that are ideal for training, competency testing, validation and R&D studies. To learn more, visit www.innogenomics.com

integenX®

Booth number: **12**
Contact person: Elaine Julian, PhD
Telephone: +49 15 253 904 830
E-mail: elainej@integenx.com
Website: www.integenx.com

Visit the market leader in Rapid DNA, to discover how the RapidHIT® system enhances community safety with a 2 hour turnaround for human identification. Forensic labs can run reference samples immediately, freeing scientists to focus their expertise on challenging samples. Law enforcement can clear or confirm suspects while in custody.

검찰
PROSECUTION SERVICE

Booth number: **19**
Contact person: Seung Hwan Lee (Mr.), Hye Hyun Oh (Mrs.)
Telephone: +82-2-3480-3730
E-mail: shlee@spo.go.kr water@spo.go.kr
Website: http://www.spo.go.kr/eng/index.jsp

The 27th ISFG Congress, which lasted for more than half of a century, will be held in the Republic of Korea for the first time in Asian countries under the catch phrase "Bridging East and West." The Supreme Prosecutors' Office will play a main role in preparing for the Congress in cooperation with

other relevant national agencies or academia. It is a great pleasure for us to invite all the attendees of the Congress to the booth where you can look around about Korea's forensic genetics. Please visit us to share your invaluable ideas about the topics of the Congress. You can also vote for social programs which you are interested in. Don't forget to get a small gift after a survey!

Booth number: **20**
Contact person: Toby Hampshire
Telephone: +44 (0)20 8943 7000
E-mail: paradna@lgcgroup.com
Website: paradna.lgcforensics.com

LGC ParaDNA® Systems generate DNA profile information in just 75 minutes, and offer free software to search/compare profiles from sample types including blood, saliva, semen and touch DNA. Designed as a rapid screening/triage tool, this highly portable platform complements existing forensic methods, as material processed using the ParaDNA Sample Collector can still be sent for full STR analysis. LGC is an international life sciences measurement and testing company, building leading positions in sustainably growing markets. Our offering is underpinned by our heritage and expertise in regulation, accreditation and standard setting. We provide reference materials, genomics solutions and analytical testing products and services, based on our innovations and our own intellectual property. We work with customers in the pharmaceuticals, agricultural biotechnology, food, environment, government, academic, security and sports sectors to achieve excellence in investigative, diagnostic and measurement science.

Booth number: **11**
Contact person: Nicole Siffling
Telephone: +49-621-8501283
E-mail: Nicole.siffling@promega.com
Website: www.promega.com

With more than 1.400 employees Promega belongs to one of the five global acting Life Science research companies.

Originally, founded in 1978 in Madison, Wisconsin, USA, Promega produces products covering the fields of genomics, protein analysis and expression, cellular analysis, drug discovery and genetic identity, Promega is a global leader in providing innovative solutions and technical support to life scientists in academic, industrial and government settings. Promega products are used by life scientists who are asking fundamental questions about biological processes as well as by scientists who are applying scientific knowledge to diagnose and treat diseases, discover new therapeutics, and use genetics and DNA testing for human identification. Promega has branches in 16 countries and more than 50 global distributors serving 100 countries.

QIAGEN

Booth number: **8**
Contact person: Dora Quest
Telephone: +492103-29-22000
Email: events@qiagen.com
Website: http://www.qiagen.com

QIAGEN is the leading global provider of Sample to Insight solutions to transform biological materials into valuable molecular insights. QIAGEN sample technologies isolate and process DNA, RNA and proteins from blood, tissue and other materials. Assay technologies make these biomolecules visible and ready for analysis. Bioinformatics software and knowledge bases interpret data to report relevant, actionable insights. Automation solutions tie these together in seamless and cost-effective molecular testing workflows. QIAGEN provides these workflows to more than 500,000 customers around the world in Molecular Diagnostics (human healthcare), Applied Testing (forensics, veterinary testing and food safety), Pharma (pharmaceutical and biotechnology companies) and Academia (life sciences research).

Qualitype

Booth number: **9**
Contact person: Dr. Frank Götz, CEO & Head of Sales
Telephone: +49 351 8838 2800

E-mail: f.goetz@qualitype.de
Website: www.qualitype.de

Qualitype GmbH has over one decade of experience in the area of forensics and software development. As a bioinformatics company we develop software concepts contributing safety and efficiency in data management in forensic examination. With state-of-the-art technology and excellent trained staff Qualitype meets the manifold demands and challenges in biotechnology and bioinformatics. Qualitype GmbH is experienced in the realization of projects of various scale and complexity for many years. Besides standardized software products, our services range from innovative concepts to the development of efficient solutions and their successful implementation. Additionally we also offer consulting, training and support for all our business areas.

SERATEC®
Gesellschaft für Biotechnologie mbH

Booth number: **15**
Contact person: Alexander Griberman
Telephone: +49 (0)551 504800
E-mail: contact@seratec.com
Website: www.seratec.com

Company profile: SERATEC develops and manufactures test kits for the rapid detection of human bodily fluids: seminal fluid, blood and saliva. We offer both laboratory and crime scene kits as well as reader devices for an objective result documentation. Extensively validated, field-proven and at reasonable cost.

silicon biosystems
A MENARINI GROUP COMPANY

Booth number: **7**
Contact person: Massimo Scrobogna
Telephone: 0039 370 3344 705
E-mail: mscrobogna@siliconbiosystems.com
Website: www.siliconbiosystems.com

With offices in Bologna, and San Diego, Silicon Biosystems has developed an instrument for cell and molecular biology, which can sort, manipulate, and collect both individual and groups of cells from a variety of samples (cancer/prenatal/forensic). The technology developed has a particular focus on resolving heterogeneity in biological mixtures of forensic cases and biological evidences. By utilizing a proprietary electronic chip-based microfluidic cartridge and microscopic

image analysis, the DEPArray™ system can recover as little as one single cell from a suspension of tens of thousands of cells with 100% purity, allowing for a range of downstream molecular analyses.

STACSDNA
Sample Tracking and Control Software for DNA Labs

Booth number: **17**
Contact person: Heather MacIntosh
Telephone: 1-613-274-7822 x 20015
E-mail: heather.macintosh@stacsdna.com
Website: www.stacsdna.com

STACS DNA delivers sample tracking and control software specifically designed for forensic DNA casework and database labs. Proven to increase productivity by more than 40% while meeting all accreditation standards–with no new hiring, and no need to acquire or replace your current LIMS. Since 2000, STACS DNA's solutions address the shortcomings of many current LIMS, giving you an automatic audit trail, DNA-specific workflows, integrated systems and instrumentation, improved data quality, automatic grant tracking and unmatched service. Customers include the FBI, RCMP, U.S. Army and the largest state labs.

STRMIX.
RESOLVE MORE DNA MIXTURES.
http://STRMIX.esr.cri.nz

NicheVision
Forensics, LLC

Booth number: **3**
Contact person: Bjorn Sutherland / Vic Meles
Telephone: +64276127378 /+1-330-252-2711
E-mail: bjorn.sutherland@esr.cri.nz
Vic@NicheVision.com
Website: strmix.esr.cri.nz
www.nichevision.com

STRmix™ is expert forensic software that can resolve previously unresolvable complex mixed DNA profiles. It uses a world leading, fully continuous approach for DNA profile interpretation. NicheVision, Inc develops software and integrated solutions automating normally tedious and time-consuming laboratory tasks to accomplish them faster, with less stress, and greater accuracy.

ThermoFisher SCIENTIFIC

Booth number: **1**
Contact person: Xavier Cristina
Telephone: +34 659 46 61 97
E-mail: Xavier.cristina@thermofisher.com
Website: lifetechnologies.com/isfg2015

Applied Biosystems™ products harness the power of science to transform lives. A member of the Thermo Fisher Scientific family of brands, our instruments, everyday tools, and services offer high-quality, innovative life science solutions for every lab and application. As a worldwide leader in forensics, we deliver some of the most comprehensively validated products, expertise, and application support available to the human identification community. From our trusted reagents, through high-performance genetic analyzers and software, to in-depth training and on-site technical assistance, our integrated systems work together to help maximize your productivity and enable your success. For more information, go to lifetechnologies.com/hid

QIAGEN

Justice after 26 years

A man who protested his innocence for 26 long years leaves his prison cell for freedom, when conclusive DNA evidence proves that someone else had committed the decades-old murder. Today, DNA evidence is making sure that justice is served. That's why we've developed **Investigator® 24plex STR Kits**, the only CODIS/ESS kits with a unique **Quality Sensor**. This internal performance control provides you with more information about your sample quality than ever before, allowing you to present your evidence in court with confidence.

Investigator 24plex – for justice today!
See www.qiagen.com/24plex or visit us at our booth 8

Sample to Insight

PROGRAM

Tuesday, September 1, 2015

19.00 - 22.00 Opening Ceremony (Large Hall)

Official welcome

ISFG Scientific Prize Lecture:
Peter Gill - Origins of modern forensic genetics

Concert: Marcin Wyrostek & Tango Corazon

Get-Together Party (-1 level)

Wednesday, September 2, 2015

8.45 - 9.00 Opening Welcome (Large Hall)

9.00 - 9.45 **Plenary lecture** (Large Hall)

Bruce Weir
SNPs and SNVs in forensic science
Chairs: *Walther Parson & Christopher Phillips*

9.45 - 12.15 Oral Session (Large Hall)

9.45 - 10.00 *Kenneth Kidd*
Genetic markers for massively parallel sequencing in forensics

10.00 - 10.15 *Weibo Liang*
Using new genetic marker microhaplotype for forensics based on next generation sequencing platform

10.15 - 10.30 *Lakshmi Chaitanya*
Simultaneous analysis of hundreds of genetic markers for multiple forensic purposes via massively parallel sequencing

10.30 - 10.45 *Sally-Ann Harbison*
A forensic MPS custom primer panel

10.45 - 11.00 *Andreas Tillmar*
A SNP panel for identity and relationship testing using next generation sequencing technology

11.00 - 11.30 Coffee (-1 level) & Posters (Exhibition Room, Seminar Room)

11.30 - 11.45 *David Ballard*
Complex mixture interpretation using massively parallel sequencing

11.45 - 12.00 *Kristiaan van der Gaag*
Massive parallel sequencing of autosomal short tandem repeats and SNPs, the next level of forensic mixture analysis

12.00 - 12.15 *Katherine Gettings*
The next dimension in STR sequencing: polymorphisms in flanking regions and their allelic associations

12.15 - 13.00 **Panel discussion** (Large Hall)

Walther Parson, Christopher Phillips, Katherine Gettings, Peter de Knijff, David Ballard, Bruce Budowle, Niels Morling
Sequencing based STR nomenclature

13.00 - 14.30 Lunch (-1 level) & Company Symposium (Medium Hall A & B)

14.30 - 15.15 **Plenary lecture** (Large Hall)

Chris Tyler-Smith
Human Y chromosome variation from whole-genome resequencing
Chairs: *Leonor Gusmao & Lutz Roewer*

15.15 - 18.00 **Oral Session** (Large Hall)

15.15 - 15.30 *Sabine Siegert*
Equivocation-based Y-STR marker selection at the population level

15.30 - 15.45 *Tamyra Moretti*
The interpretation of Y chromosome mixtures

15.45 - 16.00 *Sascha Willuweit*
Simulation of Y-STR frequentist based mixture- and kinship-analysis

16.00 - 17.00 Coffee (-1 level) & Posters (Exhibition Room, Seminar Room)

17.00 - 17.15 *Mayra Eduardoff*
Picking DNA from the ashes: a method for mtDNA sequencing of highly compromised DNA samples

17.15 - 17.30 *Cassandra Calloway*
Whole mitochondrial genome sequencing using probe capture enrichment and Illumina next-generation sequencing for analysis of mixtures and degraded DNA

17.30 - 17.45 *Jennifer McElhoe*
Low-level heteroplasmy detection on a MiSeq for mtDNA

17.45 - 18.00 *Ana Goios*
Evaluation of heteroplasmy detection in the Ion Torrent PGM

18.00 - 19.00 ISFG Working Group Meetings

20.00 - 23.00 Gala Dinner

Thursday, September 3, 2015

9.00 - 9.45 **Plenary lecture** (Large Hall)

Manel Esteller
Epigenetics in health and disease
Chairs: *Wojciech Branicki & Manfred Kayser*

9.45 - 13.00 **Oral Session** (Large Hall)

9.45 - 10.00 *Athina Vidaki*
DNA methylation-based age prediction using artificial neural networks and next generation sequencing

10.00 - 10.15 *Ana Freire-Aradas*
Inference of individual age based on DNA methylation analysis with sequenom epityper

10.15 - 10.30 *Hwan Young Lee*
DNA methylation and age prediction in semen

10.30 - 11.30 Coffee (-1 level), Posters (Exhibition Room, Seminar Room) & Company Symposium (Medium Hall A&B)

11.30 - 11.45 *Rafał Płoski*
Search for markers of human age with next generation sequencing

11.45 - 12.00 *Bram Bekaert*
A selective set of DNA-methylation markers for age determination of blood and teeth samples

12.00 - 12.15 *Ewelina Pośpiech*
HAIR genoType: forensic relevance of DNA variants associated with human hair morphology

12.15 - 12.30 *Theresa E. Gross*
Inter-laboratory evaluation of the Euroforgen global AIM-SNP set by next generation sequencing using the Ion PGMTM

12.30 - 12.45 *Jeppe Dyrberg Andersen*
The genetics of skin and eye colour and inference of ancestry in an admixed Brazilian population

12.45 - 13.00 *Susan Walsh*
The HIrisPlex-S system: combined DNA prediction of eye, hair and skin colour

13.00 - 14.30 Lunch (-1 level) & Company Symposium (Medium Hall A & B)

14.30 - 15.15 Plenary lecture (Large Hall)

Robin Williams
When global science meets local legality: deliberating and regulating forensic genetics
Chairs: *Peter Schneider & Tomasz Kupiec*

15.15 - 18.00 Oral Session (Large Hall)

15.15 - 15.30 *Oriola Sallavaci*
Cross border exchange of forensic DNA and human rights protection

15.30 - 15.45 *Tim Schellberg & Bruce Budowle*
Implementation of NGS will ultimately lead to significant expansion of the core loci: An evaluation of the identity benefits and policy/legal issues

15.45 - 16.00 *Lourdes Prieto*
The impact of the statistical evaluation of the DNA test in judicial sentences in Spain

16.00 - 17.00 Coffee (-1 level) & Posters (Exhibition Room, Seminar Room)

17.00 - 17.15 *Karolina Lech*
Towards molecular alibi testing from forensic stains: investigating mRNA markers for estimating blood-deposition time

17.15 - 17.30 *Rachel Fleming*
Body fluid identification using new transcriptomics technology

17.30 - 17.45 *Jack Ballantyne*
Targeted multiplexed next generation RNA sequencing assay for tissue source determination of forensic samples

17.45 - 18.00 *Marielle Vennemann*
Analysis of tissue specific methylation in forensics: the problem of bislufite conversion

18.00 - 19.00 **ISFG General Assembly** (Large Hall)

Friday, September 4, 2015

9.00 - 9.45 **Plenary lecture** (Large Hall)

Tomasz Grzybowski
Mitochondrial portrait of Slavic – speaking populations as revealed by complete genome analyses
Chairs: *Niels Morling & Denise Syndercombe-Court*

9.45 - 13.00 **Oral Session** (Large Hall)

9.45 - 10.00 *Krzysztof Rębała*
Comprehensive analysis of genetic variation of forensically relevant STR markers in Slavic populations

10.00 - 10.15 *Marta Kuś*
Examples of genetic identification of victims of totalitarian regimes in Poland

10.15 - 10.30 *Maarten Kruijver*
Beyond Balding-Nichols: inferring subpopulations in a forensic DNA database using a latent variable approach

10.30 - 11.30 Coffee (-1 level) & Posters (Exhibition Room, Seminar Room)

11.30 - 11.45 *Corina Benschop*
Implementing and validating interpretation software in forensic DNA casework: the LRmix studio experience

11.45 - 12.00 *Duncan Taylor*
Using Hd true tests to inform on model performance and address adventitious matching

12.00 - 12.15 *Oyvind Bleka*
EuroForMix: An open source software based on a continuous model for evaluating complex STR DNA profiles with artefacts

12.15 - 12.30 *Charles Brenner*
Continuous, fast, and thorough mixture analysis

12.30 - 12.45 *Catherine McGovern*
Interpreting mixed DNA profiles considering a range in the assigned number of contributors

12.45 - 13.00 *Klaas Slooten*
Distinguishing mixture donors from their relatives

13.00 - 14.30 Lunch (-1 level) & Company Symposium (Medium Hall A & B)

14.30 - 17.30 Oral Session (Large Hall)

Chairs: *Roland van Oorschot & Titia Sijen*

14.30 - 14.45 *Margreet van den Berge*
DNA and RNA profiling of human cell material residing on public and private objects and after activity scenario's

14.45 - 15.00 *Fabio Oldoni*
Exploring the relative DNA contribution of first and second object's users on mock touch DNA mixtures

15.00 - 15.15 *Carlo Robino*
A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers

15.15 - 15.30 *Craig Davies*
Assessing primary, secondary and tertiary DNA transfer using the promega ESI-17 fast PCR chemistry

15.30 - 15.45 *Georgina Meakin*
The deposition and persistence of indirectly-transferred DNA on regularly-used knives

15.45 - 16.00 *Lydie Samie*
Stabbing and DNA transfer

16.00 - 16.30 Coffee (-1 level)

16.30 - 16.45 *Cintia Alves*
Results of the GHEP-ISFG collaborative exercise for the taxonomic identification of forensic samples using the spindel method

16.45 - 17.00 *Sorelle Bowman*
Species identification using high resolution melting (HRM) analysis with random forest classification

17.00 - 17.15 *Barbara Karolina Zajac*
New approaches in forensic entomology - NGS technology for the identification of pupal age related molecular markers

17.15 - 17.30 *Adrian Linacre*
Robust and reproducible DNA typing of soils

20:00 - 00.00 Congress Dinner

Saturday, September 5, 2015

9.00 - 11.30 **Oral Session** (Large Hall)

Chairs: *Mechthild Prinz & Adrian Linacre*

9.00 - 9.45 *John Butler*
Reviewing scientific literature

9.45 - 10.00 *Carlos M. Vullo*
GHEP collaborative simulated exercise for DVI/MPI: lessons learned on large scale profile database comparisons

10.00 - 10.15 *Uta-Dorothee Immel*
Quality exercise in Disaster Victim Identification

10.15 - 10.30 *Daniel Vanek*
Factors influencing the reliability of the bone sample DNA typing results

10.30 - 10.45 Coffee (-1 level)

10.45 - 11.00 *Douglas Hares*
Implementation of the expanded CODIS core loci in the United States

11.00 - 11.15 *Ulises Toscanini*
Analysis of uni and bi-parental markers of mixture samples: lessons from the 22nd GHEP-ISFG intercomparison exercise

11.15 - 11.30 *Michael Coble*
Inter and intra-variation observed from a NIST interlaboratory study on DNA mixture interpretation in the U.S. (MIX13)

11.30 - 12.00 **Closing Ceremony and Coffee**

ORAL ABSTRACTS

No.	*Presenting Author*	*Title*
1	Peter Gill	Origins of modern forensic genetics
2	Bruce Weir	SNP's and SNV's in Forensic Science
3	Kenneth Kidd	Genetic markers for massively parallel sequencing in forensics
4	Weibo Liang	Using new genetic marker microhaplotype for forensics based on Next Generation Sequencing platform
5	Lakshmi Chaitanya	Simultaneous analysis of hundreds of genetic markers for multiple forensic purposes via massively parallel sequencing
6	Sally-Ann Harbison	A forensic MPS custom primer panel
7	Andreas Tillmar	A SNP panel for identity and relationship testing using next generation sequencing technology
8	David Ballard	Complex mixture interpretation using massively parallel sequencing
9	Kristiaan van der Gaag	Massive parallel sequencing of autosomal short tandem repeats and SNP's, the next level of forensic mixture analysis
10	Katherine Gettings	The next dimension in STR sequencing: polymorphisms in flanking regions and their allelic associations
11	Christopher Tyler-Smith	Human Y chromosome variation from whole-genome resequencing
12	Sabine Siegert	Equivocation-based Y-STR marker selection at the population level
13	Tamyra Moretti	The interpretation of Y chromosome mixtures
14	Sascha Willuweit	Simulation of Y-STR frequentist based mixture- and kinship-analysis
15	Mayra Eduardoff	Picking DNA from the ashes: a method for mtDNA sequencing of highly compromised DNA samples
16	Cassandra Calloway	Whole mitochondrial genome sequencing using probe capture enrichment and illumina next-generation sequencing for analysis of mixtures and degraded DNA
17	Jennifer McElhoe	Low-level heteroplasmy detection on a MiSeq for mtDNA
18	Ana Goios	Evaluation of heteroplasmy detection in the Ion Torrent PGM
19	Manel Esteller	Epigenetics in health and disease
20	Athina Vidaki	Dna methylation-based age prediction using artificial neural networks and next generation sequencing

21	Ana Freire-Aradas	Inference of individual age based on DNA methylation analysis with sequenom epityper
22	Hwan Young Lee	DNA methylation and age prediction in semen
23	Rafał Płoski	Search for markers of human age with next generation sequencing (ngs)
24	Bram Bekaert	A selective set of DNA-methylation markers for age determination of blood and teeth samples
25	Ewelina Pośpiech	HAIR genoType: forensic relevance of DNA variants associated with human hair morphology
26	Theresa E. Gross	Inter-laboratory evaluation of the Euroforgen global AIM-SNP set by next generation sequencing using the ion PGM
27	Jeppe Dyrberg Andersen	The genetics of skin and eye colour and inference of ancestry in an admixed Brazilian population
28	Susan Walsh	The HIrisPlex-s system: combined DNA prediction of eye, hair and skin colour
29	Robin Williams	When global science meets local legality: deliberating and regulating forensic genetics
30	Oriola Sallavaci	Cross border exchange of forensic DNA and human rights protection
31	T. Schellberg & B. Budowle	Implementation of NGS will ultimately lead to significant expansion of the core loci: An evaluation of the identity benefits and policy/legal issues
32	Lourdes Prieto	The impact of the statistical evaluation of the DNA test in judicial sentences in Spain
33	Karolina Lech	Towards molecular alibi testing from forensic stains: investigating mRNA markers for estimating blood-deposition time
34	Rachel Fleming	Body fluid identification using new transcriptomics technology
35	Jack Ballantyne	Targeted multiplexed next generation RNA sequencing assay for tissue source determination of forensic samples
36	Marielle Vennemann	Analysis of tissue specific methylation in forensics: the problem of bislufite conversion
37	Tomasz Grzybowski	Mitochondrial portrait of Slavic – speaking populations as revealed by complete genome analyses
38	Krzysztof Rębala	Comprehensive analysis of genetic variation of forensically relevant STR markers in Slavic populations
39	Marta Kuś	Examples of genetic identification of victims of totalitarian regimes in Poland
40	Maarten Kruijver	Beyond Balding-Nichols: inferring subpopulations in a forensic DNA database using a latent variable approach
41	Corina Benschop	Implementing and validating interpretation software in forensic DNA casework: the LRmix studio experience
42	Duncan Taylor	Using Hd true tests to inform on model performance and address adventitious matching
43	Oyvind Bleka	EuroForMix: An open source software based on a continuous model for evaluating complex STR DNA profiles with artefacts
44	Charles Brenner	Continuous, fast, and thorough mixture analysis

45	Catherine McGovern	Interpreting mixed DNA profiles considering a range in the assigned number of contributors
46	Klaas Slooten	Distinguishing mixture donors from their relatives
47	Margreet van den Berge	DNA and RNA profiling of human cell material residing on public and private objects and after activity scenario's
48	Fabio Oldoni	Exploring the relative DNA contribution of first and second object's users on mock touch DNA mixtures
49	Carlo Robino	A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers
50	Craig Davies	Assessing primary, secondary and tertiary DNA transfer using the promega ESI-17 fast PCR chemistry
51	Georgina Meakin	The deposition and persistence of indirectly-transferred DNA on regularly-used knives
52	Lydie Samie	Stabbing and DNA Transfer
53	Cintia Alves	Results of the GHEP-ISFG collaborative exercise for the taxonomic identification of forensic samples using the spindel method
54	Sorelle Bowman	Species identification using high resolution melting (HRM) analysis with random forest classification
55	Barbara Karolina Zajac	New approaches in forensic entomology - NGS technology for the identification of pupal age related molecular markers
56	Adrian Linacre	Robust and reproducible DNA typing of soils
57	John Butler	Reviewing scientific literature
58	Carlos M.Vullo	GHEP collaborative simulated exercise for DVI / MPI: lessons learned on large scale profile database comparisons
59	Uta-Dorothee Immel	Quality exercise in Disaster Victim Identification
60	Daniel Vanek	Factors influencing the reliability of the bone sample DNA typing results
61	Douglas Hares	Implementation of the expanded CODIS core loci in the United States
62	Ulises Toscanini	Analysis of uni and bi-parental markers of mixture samples: lessons from the 22nd GHEP-ISFG intercomparison exercise
63	Michael Coble	Inter and intra-variation observed from a NIST interlaboratory study on DNA mixture interpretation in the U.S. (MIX13)

1. ORIGINS OF MODERN FORENSIC GENETICS

Gill P.

ISFG Scientific Prize Lecture

Norwegian Institute of Public Health, Oslo, Norway
Department of Forensic Medicine, University of Oslo, Norway

This year marks the 30th anniversary of the initial demonstration of DNA profiling for aged forensic material that followed from the initial discovery of 'DNA fingerprinting' by Alec Jeffreys in the previous year. The first prosecution was the 'Pitchfork' case in 1987. This in turn led to the first mass-screen of 5000 people from three Leicestershire villages (UK) - a significant undertaking that established the principle of database-searches. The analysis of the Romanov family (1993-4) was one of the first historical mysteries solved by DNA profiling - single reaction STRs and mt. DNA sequencing were used to analyse samples that were highly degraded. The case was controversial at the time, but it laid the basis for low-template analysis. Multiplexing was developed shortly afterwards and the first example used in casework comprised just 4 loci and a DP of 10,000. The success of STR analysis to analyse victims of the Waco disaster was pivotal to the subsequent universal adoption of STRs by the forensic community. This in turn led directly to the development of SGM (6 loci and DP of 1 in 50 million) which was used to functionalise the first national DNA database in the world. Although significant progress had been made to develop the underlying theory to interpret complex STR mixtures by the year 2000, it is only recently that new methods have begun to become adopted more widely. Today, the most serious challenges are related to interpretation of minute quantities of DNA evidence recovered from casework.

2. SNPS AND SNVS IN FORENSIC SCIENCE

Weir B.

Department of Biostatistics, University of Washington, USA

DNA sequencing has long been the basis of forensic mitochondrial typing, but the potential for using autosomal single nucleotide variants (SNVs) has the potential for being as disruptive in forensic genetics as it has been for other areas of human genetics. Although the $1,000 genome is not yet a widespread reality, there are several large-scale whole-genome projects underway. Chip-based single-nucleotide polymorphism (SNP) typing is now well established and the availability of the 1000 Genomes reference panel is allowing 30-million-SNP profiles to be generated directly or by imputation for large numbers of people.

The use of next-generation sequencing is already having some impact, from being able to distinguish the profile of one identical twin from that of another, to uncovering variation among STR alleles of the same length, to identifying the tissue of origin of a sample, to allowing precision of ancestry determination or phenotypic prediction. The field is moving so quickly that the reliance on capillary-electrophoretic determination of short tandem repeat (STR) profiles may no longer be as assured as it was even a year ago.

This talk will review current forensic applications of SNP and SNV profiles, with an emphasis on the statistical challenges posed by genetic data that have greater breadth than those previously available to forensic scientists but also that have greater inter- and intra-individual variation and greater error rates. The focus is moving from analyses of single-location variants to consideration of constellations of genetic signals. There is enormous potential for human identification.

3. GENETIC MARKERS FOR MASSIVELY PARALLEL SEQUENCING IN FORENSICS

Kidd K. K.[1], Speed W. C.[1], Wootton S.[2], Lagace R.[2], Langit R.[2], Haigh E.[1], Chang J.[2], Pakstis A. J.[1]

[1] Department of Genetics, Yale University School of Medicine, New Haven, CT, USA
[2] Human Identification Group, Thermo Fisher Scientific, So. San Francisco, CA, USA

Massively parallel sequencing (MPS, aka NGS) has arrived for forensics and is revolutionizing the field. MPS makes information for existing STRPs

more informative and can include in a multiplex existing forensic SNP panels for information on ancestry and phenotype, types of information that forensic STRPs do not yield. MPS also makes possible microhaplotypes: small segments of DNA (<300bp) with two or more SNPs defining three or more haplotypes. Because a single sequence read can cover the expanse of the microhaplotype, these loci become phase-known codominant systems, providing much more information than a single SNP for the same effort. We have screened several hundred potential microhaplotypes and tested over 150 of the best (by various criteria) on our set of 55 populations. The majority of these microhaplotypes appear to be useful in forensics for one or more purposes. Screening of databases for candidates has focused on detecting family relationships, and/or identifying and deconvoluting mixtures of DNA from two or more individuals in a forensic sample. We have identified 86 microhaplotype loci for characterizing a mixture in a forensic sample and for identifying distant biological relationships. Large numbers of these loci can be multiplexed easily making them extremely powerful markers for forensic investigation. These microhaplotypes also allow estimation of biogeographic ancestry as well as several published AIM panels of SNPs. Microhaplotype markers will become an important, if not the major, component of future forensic markers as MPS becomes the primary technology in DNA forensics.

4. USING NEW GENETIC MARKER MICROHAPLOTYPE FOR FORENSICS BASED ON NEXT GENERATION SEQUENCING PLATFORM

Zhu J.[1], Zhou N.[3,4], Jiang Y.[1], Wang L.[1], He W.[1], Peng D.[1], Su Q.[1], Mao J.[2], Wang H.[2], Liang W.[1,*] and Zhang L.[1,*]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China
[3] College of life Sciences, Sichuan University, Chengdu 610041, Sichuan, China
[4] Bio-resources Key Laboratory of Minister of Education, Sichuan University, Chengdu 610041, Sichuan, China

liangweibo@scu.edu.cn

Microhaplotype is a kind of haplotype within 500 bp in size, including several SNPs linked together. Dr. Kenneth K. Kidd has screened some microhaplotypes from the HGDP dataset and the HapMap dataset and estimated the haplotype frequency by PHASE version 2.1.1 after genotyping the SNPs with TaqMan. But we think the single chain sequencing is the best method to profile a microhaplotype because the typing of a haplotype needs to be truly sequenced when the family is not present, and the NGS like Illumina Mi-Seq meet this demand very well. Because of no recombination happened in microhaplotype, it will be very hard to find one with many alleles. We have screened the microhaplotype from UCSC genome browser and gene bank of NCBI with the criterion of as follows. 1, the size should be shorter than 450 bp and it is depends on the read length of sequencing method. 2, the MAF of SNPs in the microhaplotype should be different from each other and the difference should be as large as possible. 3, the MAF should be investigated from at least 50 individuals, such as 1000 genome project. Based on these criterions, one microhaplotype named L8C2A including rs17720794, rs74599018 and rs16908737 with an extent of 164bp has been profiled from 18 individuals and 6 father/mother-son pairs. 3 alleles of 0.31, 0.33, 0.36 and 6 genotypes has been found. The DP of L8C2A is 0.8086 and no mutation or recombination was found in the father/mother-son pairs.

5. SIMULTANEOUS ANALYSIS OF HUNDREDS OF GENETIC MARKERS FOR MULTIPLE FORENSIC PURPOSES VIA MASSIVELY PARALLEL SEQUENCING

Chaitanya L.[1], Ralf A.,[1], van Oven M.[1], Zubakov D.[1], Kokmeijer I.[1], Rajagopalan N.[2], Calandro L.[2], Wootton S.[2], Langit R.[2], Chang C.[2], Chang J., Lagace R.[2], Kayser M.[1]

[1] Department of Forensic Molecular Biology, Erasmus MC University Medical Center, Rotterdam, Rotterdam, The Netherlands

[2] Thermo Fisher Scientific, South San Francisco, CA, USA

v.subramanyamreddy@erasmusmc.nl

Massively parallel sequencing (MPS, or next-generation sequencing, NGS) allows targeted multiplexing of large numbers of DNA/RNA markers. This is relevant for forensic cases where the evidence material is highly limited not allowing various independent tests, and/or when a large number of markers are required for maximizing the forensically relevant information. Here, we investigate the suitability and capacity of the Ion Torrent Personal Genome Machine (PGM), combined with the AmpliSeq pipeline, for targeted multiplexing of large numbers of biomarkers for multiple forensic purposes. Recently, we introduced a PGM tool based on short amplicons for parallel analysis of >530 Y-chromosome SNPs covering the entire Y-tree that allows classification of >430 worldwide Y haplogroups for ultra-high-resolution paternal lineage and ancestry inference. Now we also developed a PGM tiling approach using 161 short overlapping amplicons for complete mitochondrial genome sequencing that provides maximum-resolution maternal lineage and ancestry inference. In a first step to combine multiple biomarkers for multiple forensic purposes, we additionally developed a PGM system for simultaneous analysis of autosomal STRs, the amelogenin locus, and mRNA markers specific for all forensically relevant body tissues for simultaneous individual identification and tissue identification. As we demonstrate, combining various large marker sets including these and additional ones, such as autosomal SNPs for bi-parental ancestry and admixture identification and for eye/hair/skin-color prediction, allows the development of comprehensive and efficient tools for forensic applications. Our study illustrates the unique multiplexing ability that targeted MPS/NGS in general, and the Ion Torrent PGM in particular, provides for improving forensic analysis.

References:

1. Ralf A, van Oven M, Zhong K, Kayser M. Simultaneous analysis of hundreds of Y-chromosomal SNPs for high-resolution paternal lineage classification using targeted semiconductor sequencing. Hum Mutat. 2015 Jan;36(1):151-9.

6. A FORENSIC MPS CUSTOM PRIMER PANEL

England R.[1], Liu A.[1], Stevenson K.[1], Sharma R.[1], Stacey J.[1], Tsai P.[2], Harbison S. A.[1]

[1] Institute of Environmental Science and Research, ESR Mt Albert Science Centre, Private Bag 92-021, Mt Albert, Auckland, New Zealand
[2] Bioinformatics Institute, Auckland University, Auckland, New Zealand

Ryan.England@esr.cri.nz

With the power of massively parallel sequencing it is now possible to examine thousands of sites in the genome at once. A number of commercially developed panels have recently been released, but it is also possible to design your own.

We designed and tested a custom primer pool containing 280 forensically relevant targets including the majority of STRs currently used in commercial kits, some additional autosomal, Y and X STRs, and many SNPs. The SNPs included the 120 SNPs in the Human Identification community panel (Life Technologies) as well as SNPs shown to be associated with physical characteristics and ancestry informative markers. We sequenced these targets in eight individuals, using both the Ion PGM and the Illumina MiSeq. From the results we were successfully able to determine the STR profiles of each individual. In addition to this we were able to predict the ancestry, eye, hair and skin colour of each individual. As many of these sites are known to be highly variable, we hypothesized that they may be useful for distinguishing identical twins. Four sets of twins were sequenced using the panel, and although no differences were seen between the twins, the results provided useful information in our assessment of the utility of the custom panel.

7. A SNP PANEL FOR IDENTITY AND RELATIONSHIP TESTING USING NEXT GENERATION SEQUENCING TECHNOLOGY

Grandell I.[1], Samara R.[2], Gréen H.[1,3], Tillmar A.[1,4]

[1] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden
[2] QIAGEN Sciences Inc, Frederick, MD, USA
[3] Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden
[4] Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden

andreas.tillmar@rmv.se

There is still a need for additional markers in order to increase the power to solve cases both when it comes to identity testing and in order to solve complex relationship issues. We therefore designed an assay comprising 140 autosomal SNPs and developed an analysis using next generation sequencing technology. The customized GeneRead DNASeq SNP panel consisted of forensically relevant identity SNPs from Kidd's IISNPs (88 SNPs) and from the SNP*for*ID project (52 SNPs). The SNP panel included one single amplification step followed by library preparation using the GeneRead Library Prep workflow (QIAGEN). The sequencing was performed on a MiSeq system (Illumina) and the bioinformatic analyses were performed using the software CLC cancer research workbench (CLCbio). Each SNP locus was designed to be covered by four amplicons and the assay showed to have a balanced coverage among the included loci. The heterozygous balance showed to be high (>0.7), except for one marker rs2399332. Analyses of dilutions of the 2800M Control DNA gave reproducible results down to 0.2 ng of input DNA. In addition, 50 individuals from a Swedish population were genotyped in order to further evaluate the performance of the assay and to establish genotype frequencies. In summary, the assay offers a straightforward sample-to-genotype workflow and could be useful to gain information in forensic casework, both when it comes to identity testing and in order to solve complex relationship issues.

8. COMPLEX MIXTURE INTERPRETATION USING MASSIVELY PARALLEL SEQUENCING

Ballard D. J., Devesse L., Syndercombe Court D.

Faculty of Life Sciences and Medicine, King's College London, UK

david.ballard@kcl.ac.uk

Complex DNA mixtures are often encountered during forensic casework, however interpretation of these mixtures can be problematic and contentious. Advances achieved through novel software solutions underpinned by complex statistical methodologies have shown promise to improve this analysis. Further advances with the potential to substantially aid mixture interpretation are now available due to the enhanced ability with massively parallel sequencing (MPS) to analyse not just the allele length but also the allele sequence, and hence increase allelic discrimination. We present data here from a series of 2, 3 and 4 person mixtures that have been run both with a standard capillary electrophoresis based genotyping method and with the MPS ForenSeq kit (Illumina) that provides additional identification of allele sequence variants. Freely available continuous and semi-continuous mixture interpretation models have been used to assess what benefit the knowledge of allele sequence data brings to this mixture interpretation process.

9. MASSIVE PARALLEL SEQUENCING OF AUTOSOMAL SHORT TANDEM REPEATS AND SNPS, THE NEXT LEVEL OF FORENSIC MIXTURE ANALYSIS

van der Gaag K. J., de Leeuw R. H., de Knijff P.

Department of Human Genetics, Leiden University Medical Centre, Leiden, the Netherlands

k.j.van_der_gaag@lumc.nl

Analysis of forensic traces containing DNA from multiple donors has always been challenging. With the use of Capillary Electrophoresis (CE) for analysis of Short Tandem Repeats (STRs), minor contributions down to 20% can usually be identified. However, below that threshold it becomes increasingly challenging to distinguish minor contributions from background-signal.

Recent developments in Massive Parallel Sequencing Technologies enable new possibilities for forensic DNA-analysis. These sequencing methods can reveal additional sequence-variation from the known STRs but also enable analysis of other DNA-markers for identification purposes.

To explore additional sequence variation of 17 autosomal STRs we analysed PCR-products from a prototype version of Promega's Powerseq system for sequencing 300 samples from three globally dispersed populations using the MiSeq sequencer (Illumina). In addition, several case-samples and artificially mixed DNA-samples in ratios up to 1:99 were analysed.

To complement analysis of the most complex forensic traces we developed a new kind of forensic marker, Short Hypervariable Autosomal Fragments (SHAFs). These SHAFs contain several SNPs within a very short fragment of DNA which can be amplified in one fragment using PCR size-ranges suitable for analysis of degraded DNA without suffering from PCR stutter-artifacts.

Most STRs revealed substantial additional variation at the sequence-level compared to analysis of the same samples by CE. This additional variation increases discriminating power and facilitates better deconvolution of mixed DNA-samples since some stutter can be separated from alleles. For deconvolution of the most complex mixtures, SHAFs provided the opportunity to identify minor contributions even below 1%.

10. THE NEXT DIMENSION IN STR SEQUENCING: POLYMORPHISMS IN FLANKING REGIONS AND THEIR ALLELIC ASSOCIATIONS

Gettings K. B.[1], Aponte R. A.[2] , Kiesler K. M.[1], Vallone P. M.[1]

[1] National Institute of Standards and Technology, Gaithersburg, MD, U.S.
[2] Department of Forensic Sciences, The George Washington University, Washington, DC, U.S.

katherine.gettings@nist.gov

Recent publications have shown potential gains from sequencing the repeat regions of forensic STR loci [1]. Including flanking regions in the analyzed sequence data will add to allelic diversity, could aid kinship interpretation, and may help inform nomenclature decisions. On a more practical level, this knowledge allows for improved amplification primer design and bioinformatic pipeline development. In this study, we analyzed next generation sequencing data from 22 autosomal STR loci in 183 population samples (European, African American and Hispanic) using the STRait Razor algorithm [2] with a custom configuration file which returns the entire sequenced region, as opposed to the repeat region only. All resulting sequences (N=366) were aligned within each locus, and flanking region polymorphisms were cataloged and cross-referenced to repeat region allele sequences and populations. The flanking region sequences in this dataset can be divided into six categories, based on the types of polymorphisms they contain: 1) "old" polymorphisms in haplotype, 2) "old" single polymorphisms, 3) more "recent" single polymorphisms, 4) polymorphisms associated with sequence variants, 5) rare polymorphisms, and 6) no polymorphisms. The most benefit is expected in the first category of "old" polymorphisms in haplotype, where independently inherited SNPs are frequently observed across populations and across repeat region alleles. This pattern was present for loci such as D7S820, in which case the number of alleles obtained more than triples when the flanking sequences are added. Examples from each category of flanking region polymorphism and the potential information gain, particularly for mixture samples, will be presented.

1. Gelardi C, Rockenbauer E, Dalsgaard S, Borsting C and Morling N. Second generation sequencing of three STRs D3S1358, D12S391 and D21S11 in Danes and a new nomenclature for sequenced STR alleles. Forensic Sci Int Genet. 2014;12:38-41.

2. Warshauer DH, Lin D, Hari K, Jain R, Davis C, Larue B, King JL and Budowle B. STRait Razor: a length-based forensic STR allele-calling tool for use with second generation sequencing data. Forensic Sci Int Genet. 2013;7(4):409-17.

11. HUMAN Y CHROMOSOME VARIATION FROM WHOLE-GENOME RESEQUENCING

Tyler-Smith C.

The Wellcome Trust Sanger Institute, Hinxton, Cambs. CB10 1SA, UK

Human whole-genome sequencing is proceeding on an increasing scale, often motivated by medical considerations, and is providing sequences of thousands of Y chromosomes. Useful information about copy number variation can be obtained from most of the length of the chromosome, but information about Y-SNPs and other small variants is limited to around 10 Mb, while Y-STR interpretation is further limited by the short reads of the current sequencing technologies. Large-scale datasets that are now available or being generated include Phase 3 of The 1000 Genomes Project (1244 males in five continental groups), the CEPH Human Genome Diversity Panel (623 males from worldwide populations) and the Genome Diversity in Africa Project (sampling still in progress), as well as many projects from other investigators. Despite the limitations mentioned, the number of Y variants identified exceeds 70,000, dominated by Y-SNPs. As a result, we now have a solid calibrated Y phylogeny, new insights into mutational processes, male migrations and expansions in number, but accompanied by issues of data validation, management, merging and nomenclature. My talk will review and provide updates in these areas.

12. EQUIVOCATION-BASED Y-STR MARKER SELECTION AT THE POPULATION LEVEL

Siegert S.[1], Roewer L.[2], Nothnagel M.[1]

[1] Department of Statistical Genetics and Bioinformatics, Cologne Center for Genomics, University of Cologne, Cologne, Germany
[2] Department of Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité-Universitätsmedizin Berlin, Berlin, Germany

sabine.siegert@uni-koeln.de

Short tandem repeat (STR) markers are widely and continuously used in forensic applications. However, past research has demonstrated substantial allelic association between STR markers on autosomes as well as the X and the Y chromosomes, resulting in partially redundant discriminatory information. Recently, Siegert et al. [1] introduced approaches, based on Shannon's equivocation, to measure the extent of inter-marker association and to select a maximal discriminatory set for a fixed number of markers. By application to Y-STR markers for three continental groups, they demonstrated differential redundancy patterns between these groups, consistent with different population history, and a strongly suboptimal performance of established forensic panels, including MHT, PPY12 and Yfiler. Here, we apply this framework to populations and population groups within Europe in order to elucidate potential intra-continental differences in inter-marker association patterns and contributions to discriminatory power for the 22 Y-STR markers included in the PPY23 panel. To this end, we investigated 11,501 individuals of both European ancestry and European residency originating from 68 sampling populations, logged in the YHRD database [2]. In particular, we quantified the extent of association for different parts of the continent, applied the equivocation-based marker selection procedure to obtain regionally optimal marker panels and identified markers that disproportionally provide discriminatory information. Our results may help understanding regional differences in the discriminatory power of established marker panels.

1. Siegert, S., L. Roewer, and M. Nothnagel, Shannon's equivocation for forensic Y-STR marker selection. Forensic Sci Int Genet, 2015. 16C: p. 216-225.
2. Willuweit, S. and L. Roewer, The new Y Chromosome Haplotype Reference Database. Forensic Sci Int Genet, 2015. 15: p. 43-8.

13. THE INTERPRETATION OF Y CHROMOSOME MIXTURES

Moretti T. R.[1], Myers S. P.[2], Taylor D.[3,4], Bright J. A.[5], Buckleton J. S.[5,6]

[1] Federal Bureau of Investigation, Quantico, Virginia, USA
[2] California Department of Justice, Richmond, California, USA
[3] Forensic Science South Australia, Adelaide, South Australia
[4] Flinders University, Adelaide, South Australia
[5] ESR, Auckland, New Zealand
[6] NIST, Gaithersburg, Maryland, USA

Tamyra.Moretti@ic.fbi.gov

Y STR analysis is often used to supplement autosomal analysis when the quality, quantity or complexity of the autosomal analysis does not answer key questions of importance to the case. The resultant Y STR profiles are typically low-level and mixed. To complicate matters further, Y STR profiles often exhibit considerable stochastic variation, have some novel artifacts, and have been less extensively studied.

Despite wide acceptance of Y-STR testing in the scientific and legal communities and significant advances amplification kits and Y STR population databases, only about 60% of US forensic DNA laboratories perform Y STR analysis. In 2014, the Scientific Working Group on DNA Analysis (SWGDAM) published detailed guidance on the interpretation of characteristic Y-STR features and statistical approaches for estimation of haplotype probabilities. To supplement these guidelines, the SWGDAM Y-STR Committee is currently developing guidelines for Y mixture analysis. Because of the challenges presented by Y STR profiles, they are particularly suited to interpretation using probabilistic genotyping. No software or detailed method is available that considers drop-out and drop-in. A prototype software has been developed by the Committee that addresses many of the complexities encountered in Y STR mixture interpretation and statistical assessment.

In this presentation we outline the process used and demonstrate some results from this endeavor.

14. SIMULATION OF Y-STR FREQUENTIST BASED MIXTURE- AND KINSHIP-ANALYSIS

Willuweit S., Roewer L.

Institute of Legal Medicine, Dept. of Forensic Gentics, Charité – Universitätsmedizin Berlin, Germany

sascha.willuweit@charite.de

With the arrival of new highly informative YSTR kits (PowerPlex® Y23 System, Promega Corp. and Yfiler® Plus, Life Technologies) the YHRD rapidly formed substantial high-resolution datasets. We tested the rather aged Likelihood (LR) based approaches for Y-STR mixture calculation [1,2] and kinship calculation [4,5,6], for information gain between those high-resolution dataset and the legacy datasets available (9 to 17 Y-STRs). Being unsatisfied with the outcome, we extended both approaches in different ways and did simulation runs on real data (YHRD) and artificial data (generated) to test for the impact and the validity of each extension. We present the results of those simulation runs and propose an update to both approaches.

1. Wolf A., Caliebe A., Junge O., Krawczak M. (2005), 'Forensic interpretation of Y-chromosomal DNA mixtures.', Forensic Sci Int 152(2-3), 209-13
2. Ge J, Budowle B, Chakraborty R. (2010) 'Interpreting Y chromosome STR haplotype mixture.', Leg Med (Tokyo). 12(3):137-43
3. Rolf B., Keil W., Brinkmann B., Roewer L., Fimmers R. (2001), 'Paternity testing using Y-STR haplotypes: assigning a probability for paternity in cases of mutations.', Int J Legal Med 115(1), 12-5
4. Kayser M1, Vermeulen M, Knoblauch H, Schuster H, Krawczak M, Roewer L. (2007) 'Relating two deep-rooted pedigrees from Central Germany by high-resolution Y-STR haplotyping.', Forensic Sci Int Genet. 1(2):125-8
5. Buckleton JS., Triggs CM., Walsh SJ. (2005), Forensic DNA evidence interpretation, 1st ed., CRC press

15. PICKING DNA FROM THE ASHES: A METHOD FOR MTDNA SEQUENCING OF HIGHLY COMPROMISED DNA SAMPLES

Eduardoff M.[1], Xavier C.[1], Berger C.[1], Parson W.[1,2]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[2] *Forensic Science Program, The Pennsylvania State University, PA, USA*

mayra.eduardoff@gmail.com

One of the biggest challenges in forensic genetics is the analysis of highly degraded, low level biological material. Due to their discriminatory power, STR markers serve as the preferential option for DNA analysis. However, in cases where sample material is compromised to such an extent that conventional STR typing is not possible, mitochondrial DNA (mtDNA) analysis can still offer genetic resolution. Current conventional mtDNA analysis requires PCR and sequencing of control region segments HVR-I and HVR-II at a minimum, necessitating the presence of a certain minimal size and abundance of template DNA. With the advent of Massively Parallel Sequencing technologies, alternative methods to direct PCR for targeted DNA sequencing have been developed. These methods are based on capturing selected regions of DNA with biotin labeled complementary oligonucleotides, which are then, together with their target molecule, separated from the entire DNA pool using streptavidin beads. These captured fragments are further amplified within the library preparation process. Some of these methods have been adapted for use on ancient DNA remains, proving to be a powerful and sensitive tool for DNA analysis in highly degraded, low target and high background sample environments. Here we present the adaption of a Primer Extension Capture method for MPS sequencing of mitochondrial DNA in highly compromised samples. Sample mtDNA abundance and quality were assessed using different qPCR DNA quantification approaches, targeting 39bp to 143bp mtDNA fragments. Those samples showing excessive fragmentation and negligible quantification results were analyzed by Primer Extension Capture.

16. WHOLE MITOCHONDRIAL GENOME SEQUENCING USING PROBE CAPTURE ENRICHMENT AND ILLUMINA NEXT-GENERATION SEQUENCING FOR ANALYSIS OF MIXTURES AND DEGRADED DNA

Calloway C. D., PhD[1,2], Gordon R., MS[1], Erlich H., PhD[1]

[1] *Children's Hospital Oakland Research Institute, Oakland, CA, USA*
[2] *University of California, Davis, Davis, CA, USA*

scalloway@chori.org

We have successfully developed a solution phase probe capture and Next-Generation Sequencing (NGS) method for targeted enrichment and deep sequencing of the entire mitochondrial genome for increased discrimination power. Using this probe capture NGS assay, 100% sequence coverage of the mitochondrial genome with an ~95% on target rate was achieved. The sequence read coverage achieved with this optimized probe capture method was more evenly distributed with only ~2X difference in average read depth per base across the mitochondrial genome. The input DNA amount was successfully lowered to the forensically relevant level of 10 pg demonstrating the high sensitivity of the method. This method which uses the Illumina MiSeq NGS technology also allows for ultra-deep sequencing and resolution of mixtures not possible using standard Sanger sequencing methods for mtDNA analysis. Moreover, a DNA fragmentation method using mechanical shearing (Covaris) was optimized and shown to be DNA quantity and quality independent, essential for preparation of highly degraded or limited samples often encountered in forensic cases. This optimized fragmentation method coupled with the probe capture enrichment assay can be used for analysis of degraded samples often encountered in forensic cases as well as mixtures with increased sensitivity and discrimination potential.

17. LOW-LEVEL HETEROPLASMY DETECTION ON A MISEQ FOR MTDNA

McElhoe J. A.[1], Holland M. M.[1]

[1] *The Pennsylvania State University, University Park, Pennsylvania, U.S.A.*

jam760@psu.edu

Forensic laboratories are not taking advantage of intrinsic levels of mitochondrial (mt) DNA heteroplasmy that exists in humans. While current techniques and technologies provide for reliable reporting of mtDNA haplotypes, the methods in place today do not effectively identify heteroplasmic variants (especially low-level variants), and even when heteroplasmy is observed at high levels, the information is typically not used during the investigation. The ability to identify, report, and utilize the discrimination potential of heteroplasmy will significantly enhance the value of mtDNA analysis in forensic casework. A next generation DNA sequencing (NGS) approach will allow the community to achieve this goal. Methods for sequencing mtDNA using NGS have been developed, published, and are readily available to forensic practitioners[1-2]. In addition, studies are underway to develop NGS methods for the analysis of difficult samples types (for example, hair shafts[3] and older skeletal remains). Most recently, work is underway in our laboratory to measure the rate of heteroplasmy in the control region (CR), to evaluate the transmission of heteroplasmic variants between maternal relatives and different tissue types, and to develop recommended best practices for reporting of heteroplasmy, including weight estimates. We will report on our progress with this project, on a novel approach we have developed to address low NGS read coverage in the CR, and on recent efforts to modify the NextGENe software package (in collaboration with SoftGenetics) for forensic purposes.

1. McElhoe, J.A., Holland, M.M. et. al.: Development and assessment of an optimized next-generation DNA sequencing approach for the mtgenome using the Illumina MiSeq. FSIG. 2014; 13:20-29.
2. Parson, W., Strobl, C. et. al.: Evaluation of next generation mtgenomes sequencing using the Ion Torrent Personal Genome Machine (PGM). FSIG. 2013; 7:543-549.
3. Parson, W., Huber, G. et. al.: Massively parallel sequencing of complete mitochondrial genomes from hair shaft samples. FSIG. 2015; 15:8-15.

18. EVALUATION OF HETEROPLASMY DETECTION IN THE ION TORRENT PGM

Magalhães S.[1, 2, 3], Marques S. L.[1, 2], Alves C.[1, 2], Amorim A.[1, 2, 3], Alvarez L.[1, 2], Goios A.[1, 2]

[1] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*
[2] *Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup), Porto, Portugal*
[3] *Faculdade de Ciências da Universidade do Porto, Porto, Portugal*

aalmeida@ipatimup.pt

The use of mitochondrial DNA (mtDNA) heteroplasmy in forensic genetics casework has been scarce, being limited to a few specific situations. The heteroplasmic condition is commonly disregarded in databasing, mostly due to the technical limitations of the PCR/sequencing technology.

Sanger sequencing technology is not sensitive enough to detect low-level heteroplasmy and, per se, it does not allow accurate allele quantification. Presently, however, it is possible to sequence complete mtDNA genomes while detecting and quantifying heteroplasmic mutations by Massively Parallel Sequencing (MPS). Several studies have been carried out recently regarding the threshold for heteroplasmy detection by MPS, being generally suggested that this methodology is more sensitive and accurate in evaluating heteroplasmy levels than Sanger sequencing, particularly when contamination is controlled and the amplification strategy is carefully designed.

In this work, we present the results of a sensitivity study of heteroplasmy detection and quantification with Ion Torrent PGM, using control samples

of predetermined haplotypes belonging to different haplogroups. The amplification strategy used for the PGM sequencing is based on a single long-range PCR, in order to even the proportions of the mixed bases throughout the molecule. To simulate different levels of heteroplasmy we used mixtures of the amplified control samples (amplicons) at variable proportions. The sequences obtained through the PGM are compared to those obtained by Sanger sequencing to determine heteroplasmy detection threshold with both technologies.

19. EPIGENETICS IN HEALTH AND DISEASE

Esteller M.

Institute for Biomedical Research (IDIBELL), Barcelona, Spain

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders such as neurodegenerative or cardiovascular diseases. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs. Beyond that, epigenetics is involved in the aging process and many others aspects of human biology.

20. DNA METHYLATION-BASED AGE PREDICTION USING ARTIFICIAL NEURAL NETWORKS AND NEXT GENERATION SEQUENCING

Vidaki A.[1], Ballard D.[1], Barron L.[1], Syndercombe Court D.[1]

[1] Department of Pharmacy and Forensic Science, King's College London, London, United Kingdom

athina.a.vidaki@kcl.ac.uk

The ability to estimate the age of the donor from recovered biological material at a crime scene can be of substantial value in forensic investigations. Aging is complex and associated with various molecular modifications in cells that accumulate over a person's lifetime. The aim of this study was to use age-specific DNA methylation patterns to predict age from whole blood. Based on their previously reported age coefficient in the literature, 45 age-associated CpG sites were investigated using publicly available methylation data obtained from 1156 blood samples. Multiple regression analysis revealed that a subset of 16 CpGs was capable of accurately predicting age (R^2=0.92, mean absolute error=5.3 years). Applying a generalized regression neural network, the age prediction improved (R^2=0.96, mean absolute error=3.3 years, training set and 4.4 years, blind test set). To assess its applicability, the model was further validated using an independent cohort of monozygotic twins, various diseased blood samples as well as non-blood tissues. In an attempt to create a sensitive and accurate age prediction test, a method able to quantify the methylation status of the selected 16 CpG sites was developed using Illumina's MiSeq platform. The method was validated using DNA standards of

known methylation levels and the age prediction accuracy was assessed from a set of whole blood samples. Although the resulted prediction accuracy was lower than the one obtained from the original model, it is hoped that future optimisation will improve both prediction accuracy and reproducibility.

21. INFERENCE OF INDIVIDUAL AGE BASED ON DNA METHYLATION ANALYSIS WITH SEQUENOM EPITYPER

Freire-Aradas A.[1], Phillips C.[1], Mosquera-Miguel A.[1], Girón-Santamaría L.[1], Gómez-Tato A.[2], Álvarez Dios J.[2], Casares de Cal M.[2], Pośpiech E.[3], Branicki W.[4], Carracedo Á.[1], Lareu M. V.[1]

[1] Forensic Genetics Unit, Institute of Forensic Sciences, University of Santiago de Compostela, Santiago de Compostela, Spain
[2] Faculty of Mathematics, University of Santiago de Compostela
[3] Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University
[4] Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland

ana.freire3@hotmail.com

Forensic DNA intelligence has opened the door to analysis of cases that lack DNA database entries or potential suspects. Far from placing aside such casework, the prediction of externally visible characteristics becomes a useful source of genetic information that can reveal certain physical traits of the perpetrator[1]. Additionally, individual age estimation has obvious relevance for finding out more about someone present at crime scene, as age is a clear personal characteristic that is difficult to disguise while providing key information about the DNA donor to investigators. Recent studies on epigenetic biomarkers have shown that DNA methylation changes occur throughout the human lifespan[2]. Therefore, the analysis of hyper- or hypo-methylation patterns from a biological stain will provide relevant information to help ascertain the chronological age of the donor. In the present study we have explored the DNA methylation levels of a set of CpG sites from eight genes across the human genome. Individuals of different ages were investigated using the *Sequenom EpiTYPER* system, a method that uses base specific cleavage and matrix-assisted laser desorption/ionization time-of-fight mass spectrometry (MALDI-TOF MS). The corresponding PCR primers were designed using *EpiDesigner* and the *MassArray* R package. Differences in DNA methylation levels observed versus chronological age were assessed in order to establish a prediction model. These findings are presented with assessments of the most informative loci for forensic age estimation purposes.

1. Kayser M, de Knijff P. Nat Rev Genet. 2011; 12 (3): 179-192.
2. Zbieć-Piekarska R, Spólnicka M, Kupiec T, et al. Forensic Sci Int Genet. 2015; 14: 161-167.

22. DNA METHYLATION AND AGE PREDICTION IN SEMEN

Oh Y. N., Jung S. E., Choi A., Shin K. J., Yang W. I., Lee H. Y.

Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

hylee192@yuhs.ac

To date, DNA methylation has been regarded as the most promising age-predictive biomarker. In support of this, several researchers have reported age predictive models based on the use of blood or even across a broad spectrum of tissues. However, there have been no publications that report epigenetic age signatures from semen, one of the most forensically relevant body fluids. In genome-wide DNA methylation profiles of 36 body fluids including blood, saliva, and semen, the previous age predictive models showed considerable prediction accuracy in blood and saliva but not in semen. Therefore, we selected CpG sites, whose methylation levels are strongly correlated with age from Illumina DNA methylation 450K array of 12 semen samples obtained from individuals of different ages. Then, we investigated DNA methylation changes at these CpGs in 61 additional semen samples obtained from individuals aged 20 to 73 years using methylation SNaPshot reaction. Among the selected age-related CpG candidates, outstanding age correlation was

obtained at cg06304190 in the TTC7B gene and cg06979108 in the NOX4 gene. The age-predictive linear regression model trained with the two CpGs in the TTC7B gene and the NOX4 gene showed a high correlation between the predicted age and the chronological age, with an average absolute difference of approximately 5 years. The accuracy of the model was further tested with additional semen samples. These selected epigenetic age signatures are expected to be useful for considerably accurate age estimation in the forensically relevant body fluid of semen.

23. SEARCH FOR MARKERS OF HUMAN AGE WITH NEXT GENERTAION SEQUENCING (NGS)

Kosińska J.[1], Gasperowicz P.[1], Zbieć-Piekarska R.[2], Spólnicka M.[2], Makowska Ż.[2], Stawinski P.[1], Próchenka A.[1], Parys-Proszek A.[3], Kupiec T.[3], Branicki W.[3,4], Płoski R.[1]

[1] Warsaw Medical University, Warsaw, Poland
[2] Central Forensic Laboratory of the Police, Warsaw, Poland
[3] Institute of Forensic Research, Krakow, Poland
[4] Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

rploski@wp.pl

Recent publications indicate that in humans correlation exists between age and methylation level of certain genomic loci. Whereas majority of data come from microarray studies allowing to analyze a limited fraction of genome the published correlations appear strong enough for potential forensic use. Our purpose was to search at the genomewide level for novel loci whose methylation correlates with age. We used Reduced Representation Bisulphite Sequencing (RRBS) together with NGS on Illumina HiSeq 1500 platform. We analyzed 187 male individuals classified as young (<30 years) or elderly (>50 years). On average for each sample 20 mln reads of 100 bp were generated. 0.4% of cytosines (Cs) were filtered out due to presence of SNPs influencing methylation site. For each C covered in a given sample frequency of reads indicating methylation vs. all reads was generated. Using a criterion that a given C should be covered by min. 5 reads in min. 5 samples from each group in the final data set we had > 4.5 mln Cs in CpG, 8,6 mln Cs in CpHpG and 20,4 mln Cs in CpHpH (where H denotes a different base than C). So far we focused analysis on CpG methylation. Using a Bonferroni correction 4210 sites were significantly associated with age at $P<0.05$. An alternative approach based on FDR (false discovey rate) of 0.05 revealed 111 790 age related Cs. The presented data are the largest data set available for searching of age related DNA methylation differences.

24. A SELECTIVE SET OF DNA-METHYLATION MARKERS FOR AGE DETERMINATION OF BLOOD AND TEETH SAMPLES

Bekaert B.[1,2], Kamalandua A.[1], Zapico S. C.[3], van de Voorde W.[1,2], Decorte R.[1,2]

[1] Forensic Biomedical Sciences, Department of Imaging & Pathology, KU Leuven - University of Leuven, Leuven, Belgium
[2] Department of Forensic Medicine, Laboratory of Forensic Genetics and Molecular Archaeology, University Hospitals Leuven, Leuven Belgium
[3] Department of Anthropology, Smithsonian Institution, Washington, USA

bram.bekaert@uzleuven.be

The ageing process leads to modifications in tissues and organs on a molecular level which can be used by forensic scientists to determine the age of living and deceased individuals and also from donors of biological traces. Recently, a number of papers have been published on the association of DNA methylation markers and the chronological age of blood sample donors [1]. We aimed to increase the accuracy of this method using age-associated CpG sites according to the literature [2-4]. After a pilot study to identify the most strongly associated CpG sites we analysed DNA methylation levels of 5 CpG sites in 4 genes ASPA, PDE4C, EDARADD and ELOVL2 using pyrosequencing in 208 blood samples (ages 0-91) from deceased individuals with known chronological age. Multivariate linear regression produced a Mean Absolute

Deviation (MAD) of 4.36 years with a very high correlation between predicted and chronological age (r=0.96). Subdividing this population into two bins of ages <60 and ≥60 changed the MAD to respectively 3.47 (r=0.96) and 6.16 (r=0.70) years, indicating a wider variance with increasing age. Analysing 30 dentin samples with the same set we obtained a MAD of 6.55 years (r=0.81) suggesting that other, more informative markers, might be necessary for this type of cellular matrix (manuscript in preparation). In the future we intend to extend this application to other matrices (blood stains, saliva, sperm and bone) and are in the course of developing a methyl-SNaPshot assay for age estimation to reduce time, cost and sample consumption.

1. Bacalini M.G. et al. A meta-analysis on age-associated changes in blood DNA methylation: results from an original analysis pipeline for Infinium 450k data. Aging. 2015 Jan 7(1):97-109.
2. Weidner, C.L. et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol. 2014 Feb 3;15(2):R24.
3. Garagnani P. et al. Methylation of ELOVL2 gene as a new epigenetic marker of age. Aging Cell. 2012;11(6):1132–4.
4. Zbieć-Piekarska R. et al. Examination of DNA methylation status of the ELOVL2 marker may be useful for human age prediction in forensic science. Forensic Sci Int Genet. Elsevier Ireland Ltd; 2014;14C:161–7.

25. HAIR GENOTYPE: FORENSIC RELEVANCE OF DNA VARIANTS ASSOCIATED WITH HUMAN HAIR MORPHOLOGY

Pośpiech E.[1], Marcińska M.[2], Abidi S.[3], Andersen J. D.[4], van den Berge M.[5], Carracedo Á.[6,7], Eduardoff M.[8], Freire-Aradas A.[6], Morling N.[4], Sijen T.[5], Skowron M.[9], Söchtig J.[6], Syndercombe Court D.[3], Weiler N.[5], The EUROFORGEN-NoE Consortium; Ballard D.[3], Børsting C.[4], Parson W.[8,10], Phillips C.[6], Branicki W.[1,2]

[1] Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland
[2] Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland
[3] Faculty of Life Sciences, King's College, London, United Kingdom
[4] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[5] Department of Human Biological Traces, Netherlands Forensic Institute, The Hague, The Netherlands
[6] Forensic Genetics Unit, Institute of Forensic Medicine, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain
[7] Genomic Medicine Group, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Institute of Health Carlos III, Spain
[8] Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria
[9] Department of Dermatology, Medical College of Jagiellonian University, Krakow, Poland
[10] Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania, United States of America

ewelina.pospiech@uj.edu.pl

Hair morphology is a highly variable and distinctive feature of human physical appearance worth considering in the field of Forensic DNA Phenotyping. Despite its high heritability (~90%), genetic determinants that influence hair texture remain largely undefined. Here we conducted a population association study involving 6 SNPs in 3 genes (TCHH, WNT10A, FRAS1) previously associated with hair morphology and 44 SNPs associated with male pattern baldness (MPB). The initial study involved 528 individuals from Poland. Association analyses performed on a discovery set of 428 individuals revealed the TATC haplotype (f=22.06%) in TCHH was significantly associated with straight hair with OR=1.98, 95%CI=1.37-2.88 and $p=3.06x10^{-4}$. Multivariate logistic regression confirmed association with hair morphology for rs11803731 in TCHH, rs7349332 in WNT10A and rs1268789 in FRAS1. The combined genotype (OR=2.72, $p=2.02x10^{-6}$) explained 7.9% of the total variance of hair morphology. The developed prediction model involving these 3 SNPs and the discovery set was found to predict straight hair with high sensitivity (96%) but low specificity (18%) when applied to a test set of the

remaining 100 Polish samples. Additional testing involved 146 samples from six European populations and revealed similar results (92.8% sensitivity and 18.5% specificity). The results obtained indicate the genotype involving SNPs: rs11803731-rs7349332-rs1268789, TT-CC-GG (f=4.5%) is a strong indicator of straight hair (P=87%) and gave 100% of correct results in the testing set. Analysis extended to SNPs involved in MPB also suggested potential associations with hair morphology.

26. INTER-LABORATORY EVALUATION OF THE EUROFORGEN GLOBAL AIM-SNP SET BY NEXT GENERATION SEQUENCING USING THE ION PGM

Gross T. E.[1], Eduardoff M.[2], Santos C.[3], de la Puente M.[3], Ballard D.[4], Strobl C.[2], Børsting C.[5], Morling N.[5], Syndercombe Court D.[4], Sobrino B.[6], Carracedo Á.[3,6], Parson W.[2], Phillips C.[3], Schneider P. M.[1]
and the EUROFORGEN-NoE Consortium

[1] Institute of Legal Medicine, University Hospital Cologne, Cologne, Germany
[2] Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria
[3] Forensic Genetics Unit, Institute of Legal Medicine, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain
[4] Department of Forensic and Analytical Science, King's College, London, UK
[5] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[6] Grupo de Medicina Xenómica (GMX), Faculty of Medicine, University of Santiago de Compostela

theresa.gross@uk-koeln.de

The EUROFORGEN Global AIM-SNP set designed for the Ion PGM™ system comprises 128 SNPs chosen to provide balanced differentiation of the five continental population groups of Africa, Europe, East Asia, Native America, and Oceania. In this study the Global AIM-SNP set was evaluated between four different laboratories with the focus on SNP performance, genotyping precision, sensitivity, ability to detect mixtures and ancestry differentiation. For 115 of 128 AIMs no problems were found, while no-calls or discordances were observed in 8 and 6 SNPs respectively. The very high concordance rate of 99.8% resulted from comparison of Coriell control DNA analyses to public whole-genome sequencing data from 1000 Genomes and Complete Genomics projects. Evaluations of sensitivity showed 80-97% of correct genotype calls could be obtained from 10-50 pg of input DNA. Preliminary STRUCTURE and principal component analyses with 1000 Genomes and HGDP-CEPH SNP genotypes showed good differentiation between the major continental groups1. The inclusion of genotypes from more than 500 additional samples of 14 different populations, not already covered by the above projects, extends worldwide population data coverage. This allows an assessment of the degree of differentiation achievable with the Global AIM SNP set between closely related population groups (e.g. Europe and Middle East). Dedicated allele frequencies and training sets are currently implemented in SPSmart and the online classifier Snipper to further support use of this set. Therefore, results reported here indicate the EUROFORGEN Global AIM-SNP set provides a sensitive and accurate forensic SNP assay for broadly-based ancestry prediction.

1. C. Phillips, W. Parson, B. Lundsberg et al.: Building a forensic ancestry panel from the ground up: The EUROFORGEN Global AIM-SNP set. Forensic Science International: Genetics. 2014; 11: 13–25.

27. THE GENETICS OF SKIN AND EYE COLOUR AND INFERENCE OF ANCESTRY IN AN ADMIXED BRAZILIAN POPULATION

Dyrberg Andersen J.[1], Jannuzzi J.[2], Simão F.[2], Pereira V.[1], Morling N.[1], Cavalho E.[2], Gusmão L.[2,3,4]

[1] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[2] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil

[3] IPATIMUP (Institute of Pathology and Molecular Immunology from de University of Porto), Portugal
[4] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

Jeppe.dyberg.andersen@sund.ku.dk

In the identification of persons and in certain crime cases, DNA information about externally visible characteristics can be important for the police investigators. Ancestry and specific physical traits, including eye, skin and hair colour are closely correlated. It may be argued that the prediction of ancestry will also give a prediction of eye, hair and skin colour. However, this is only true in non-admixed populations.

In this study, the eye and skin colours as well as the ancestry of an admixed Brazilian population sample of more than 400 individuals were investigated using genetic markers. A total of 28 single nucleotide polymorphisms (SNPs) in 14 different pigmentation genes were compared with quantitative measurements of skin and eye colours. The pigmentary genes included SLC24A4, SLC45A2, SLC24A5, TYR, TYRP1, OCA2, HERC2, DCT, IRF4, UGT1A, BCN2, EGFR, KITLG and ASIP. Eye colour was evaluated using the Pixel Index of the Eye (PIE)-score and skin colour was evaluated using reflectance measurements. For each individual, the proportion of European, Native American and African ancestry was estimated using 46 ancestry informative InDel loci. The correlation between SNPs and eye and skin colours will be presented. The effect of ancestry will be considered in the correlation analysis.

28. THE HIRISPLEX-S SYSTEM: COMBINED DNA PREDICTION OF EYE, HAIR AND SKIN COLOUR

Walsh S.[1], Chaitanya L.[2], Breslin K. A.[1], Kovatsi L.[3], Wojas-Pelc A.[4], Branicki W.[5, 6], Liu F.[2], Kayser M.[2]

[1] Department of Biology, Indiana University Purdue University Indianapolis (IUPUI), Indianapolis, Indiana 46202, USA.
[2] Department of Forensic Molecular Biology, Erasmus MC University Medical Centre Rotterdam, Rotterdam, The Netherlands
[3] Laboratory of Forensic Medicine & Toxicology, School of Medicine, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
[4] Department of Dermatology, Collegium Medicum of the Jagiellonian University, Kraków, Poland
[5] Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland
[6] Department of General Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University, Kraków, Poland

walshsus@iupui.edu

Forensic DNA Phenotyping (FDP) has become a fast growing topic in forensic research and practice. FDP is expected to aid police investigations by providing physical appearance information on unknown individuals when conventional DNA profiling, or other means of investigation are non-informative. Previously we developed the IrisPlex [1] and HIrisPlex [2] systems for eye and hair colour prediction from DNA. Here, we add skin colour by introducing the HIrisPlex-S system for combined eye, hair, and skin colour DNA prediction. Eye and hair colour prediction is obtained via the previously introduced single HIrisPlex SNaPshot multiplex assay targeting 24 SNPs and the eye and hair colour prediction models, while a second SNaPshot multiplex is added that targets 18 skin colour specific SNPs. For the new skin colour prediction model, a total of 36 SNPs from both multiplexes provide population specific AUC prediction accuracy values ranging from 0.76 to 1 for five categories of skin colour; very light, light, medium, medium to dark, and dark skin colour in a validation set of approx. 240 individuals from nine populations while approx. 960 individuals were used for model building. With this study, we present the HIrisPlex-S system consisting of two multiplex assays and three statistical prediction models enabling combined predictions of all three human pigmentation traits in one DNA test system. Furthermore, we assess the full performance of the system on an additional set of individuals from a US population to substantiate its future application in forensic, missing person, as well as archaeological and anthropological cases.

1. Walsh et al. Forensic Sci Int Genet. 2011,5:170-180.
2. Walsh et al. Forensic Sci Int Genet. 2013,7:98-115.

29. WHEN GLOBAL SCIENCE MEETS LOCAL LEGALITY: DELIBERATING AND REGULATING FORENSIC GENETICS

Williams R.

Northumbria University, Newcastle Upon Tyne, UK

This presentation will consider the range of social and ethical considerations that have underpinned regional and national efforts to determine the acceptable uses of forensic genetics in support of criminal justice objectives. It will pay particular attention to a common repertoire of moral concepts, ethical principles and social goals. It will note how policy-makers in different jurisdictions have foregrounded particular items from this repertoire when making decisions about what and how particular forensic genetic technologies should be routinely and exceptionally used. In the presentation I will also discuss the ways in which recent innovations in forensic genetics have come to challenge prior social, ethical and legal understandings and argue for the involvement of forensic geneticists in the anticipatory governance of such innovations. Finally, I will attend to the practice of forensic genetics, arguing that actions informed by ethically reflexive judgements are to be preferred to those which simply show allegiance to formal ethical codes.

30. CROSS BORDER EXCHANGE OF FORENSIC DNA AND HUMAN RIGHTS PROTECTION

Sallavaci O.

Anglia Ruskin University, Anglia Law School, Chelmsford, United Kingdom

In recent years many countries in the world have increased their efforts to achieve a closer transnational cooperation in combating terrorism, cross border crime and illegal immigration. In Europe, the Prüm regime was established to provide mechanisms and infrastructure to achieve these goals. Forensic DNA constitutes an important investigative and evidentiary resource in the contemporary criminal justice systems and, within the transnational cooperation framework, the exchange of DNA profiles and data acquire great relevance. A number of ethical and legal issues surround the forensic use of DNA. These issues relate to the protection of individual rights during DNA sample collection and retention, their subsequent use for investigative or evidentiary purposes and, in the context of cross-border cooperation, the transmission of data to another country. Among the rights involved are the right to physical and moral integrity, not to be subjected to degrading treatment, the right not to incriminate oneself, the right to privacy and personal data protection. Cross-border exchange of forensic DNA raises specific concerns as the protection of fundamental rights has to be assured by the domestic legislation of each country involved which in turn must be compliant to international legal provisions binding on those countries. This paper explores how such protection can be provided and how the transnational collaboration can be achieved with respect to fundamental rights in the context of the legal frameworks operating at domestic, EU and international levels.

31. IMPLEMENTATION OF NGS WILL ULTIMATELY LEAD TO SIGNIFICANT EXPANSION OF THE CORE LOCI: AN EVALUATION OF THE IDENTITY BENEFITS AND POLICY / LEGAL ISSUES

Schellberg T.[1], Budowle B.[2,3]

[1] *Gordon Thomas Honeywell Governmental Affairs, Seattle, WA, USA*
[2] *Institute of Applied Genetics, University of North Texas health Science Center, Ft Worth, TX, USA*
[3] *Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia*

bruce.budowle@unthsc.edu

There is growing realization that next generation sequencing (NGS) will one day replace CE instrumentation in the crime lab and in the interim run in parallel with CE approaches. Once a majority of a country's reference samples

are being run on NGS platforms, it is inevitable that the issue of expanding the core loci will be considered and debated. NGS has the ability to produce all relevant genetic markers and beyond in one analysis. In the pursuit of justice, proposals will surely develop to expand the databases to many more autosomal STRs, full panels of Y STRs and possibly X STRs, and SNPs. With this capability, policy makers will react. Their actions and responses will have policy implications on sensitive issues that relate to privacy. Legislation should be designed to deal with these issues so that society can benefit from the power of NGS within a sound framework. To overcome policy objections and move large scale changes in the selected loci forward, the value of enhancing investigative leads, and ultimately solving more crimes were developed. The presentation evaluates the increased benefits of expanding the core loci and its potential impact on crime solving and then describes potential risks to privacy which include novel markers such as AIMs and whole genome sequencing data that will arise with resolving twins. Policy issues and guidance are provided regarding core loci expansion proposals.

32. THE IMPACT OF THE STATISTICAL EVALUATION OF THE DNA TEST IN JUDICIAL SENTENCES IN SPAIN

Prieto L.[1], Carracedo Á.[1]

[1] Forensic Sciences Institute, University of Santiago de Compostela, Spain

lourdes.prieto@usc.es

The incorporation of new genetic markers, new technological tools and new software to statistically interpret DNA evidence have allowed us to improve on the answers that DNA analysis can give to clarify criminal cases. But not enough attention has been given to how legal professionals (judges, prosecutors and lawyers) understand and interpret DNA result.

In order to test how the meaning of DNA analysis is understood in the Spanish legal system, we have reviewed 397 judicial sentences (2013-2015) in which the DNA test was relevant. In 369 of these sentences (93%), the statistical evaluation of the DNA test is not mentioned and even sometimes sentences state directly that the genetic profile found in the evidence belongs to the accused (or the victim). Only 28 out of the 397 reviewed sentences (7%) refer to value of the evidence. Nevertheless, some of these sentences do show some problems, such as (i) misunderstanding the meaning of the frequency of a DNA profile, (ii) the lack of hypotheses to be compared when LR is used, (iii) the transposition of conditional and (iv) confusion about the distinction between the reliability of the test (the error rate) and the evaluation of the results of the test (RMP/LR). In addition, erroneously associating the presence of a DNA profile with a particular activity (e.g. sexual assault) was also detected in many of the sentences ("association fallacies")[1].

1. Gill P.: Misleading DNA evidence, Reasons for miscarriages of Justice. Academic Press, Elsevier, 1st Ed. 2014.

33. TOWARDS MOLECULAR ALIBI TESTING FROM FORENSIC STAINS: INVESTIGATING MRNA MARKERS FOR ESTIMATING BLOOD-DEPOSITION TIME

Lech K.[1], Liu F.[1], Ackermann K.[1,2], Revell V. L.[3], Skene D. J.[3], Kayser M.[1]

[1] Department of Forensic Molecular Biology, Erasmus MC University Medical Centre Rotterdam, 3015 CN Rotterdam, The Netherlands
[2] School of Chemistry and Centre of Magnetic Resonance, University of St Andrews, St Andrews, Fife, KY16 9ST, United Kingdom
[3] Centre for Chronobiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, Surrey, United Kingdom

k.lech@erasmusmc.nl

Estimating the day/night time a biological trace was left at a crime scene is forensically relevant as if possible it would allow alibi testing directly from the trace evidence, helping to link (or not) DNA-identified sample donors with crime events. Previously, we introduced two circadian hormones, melatonin and cortisol, for forensic blood-deposition timing. However, the time

information obtainable with these hormones is not perfect, partly because hormone levels can change not only due to daily rhythms. Therefore, we investigated mRNA as source of potential biomarkers for this forensic purpose. From a set of candidate genes for which we established the expression patterns in blood samples of several individuals collected under controlled conditions at 2 h intervals for 36 h, we identified several mRNA markers with statistically significant daily expression rhythms. We then used the selected mRNA markers with and without melatonin and cortisol to establish various multinomial regression models for predicting day/night time categories from the molecular data. In general, prediction models considering only mRNA markers provided less accurate time estimations than those based only on hormones. Improved prediction accuracy was observed when combining three mRNA markers *HSPA1B, MKNK2* and *THRA1* with the two hormones melatonin and cortisol. This model was favoured by our data, showing an improved performance in predicting three day/night time categories: night/early morning, morning/noon, and afternoon/evening. Therefore, for the first time, we demonstrate the promising value of rhythmically expressed mRNAs for blood-deposition timing, especially when used in combination with circadian hormones.

34. BODY FLUID IDENTIFICATION USING NEW TRANSCRIPTOMICS TECHNOLOGY

Fleming R.[1], Lin M-H.[1]

[1] *Institute of Environmental Science and Research Ltd., Auckland, New Zealand*

Rachel.Fleming@esr.cri.nz

The identification of body fluids is becoming a core capability in forensic science. Messenger RNA (mRNA) analysis is a commonly used method for body fluid identification and is used in forensic casework at ESR. It is well known that the physiological nature of mRNA makes it prone to degradation, which influences the sensitive and specific detection of RNA from forensic stains. The recent rapid development of massively parallel sequencing (MPS) technology has enabled us, with specific changes to conventional MPS methodology, to sequence significantly degraded, low abundance total RNA from forensic samples. The transcriptomics data has provided fundamental information to further characterise the RNA transcripts targeted for body fluid identification. Using this information, we have significantly improved the performance of RNA detection methods (PCR), regardless of RNA abundance and degradation levels. These new transcriptomic insights have been integrated into casework testing. In addition to total RNA, we have used MPS to identify microRNAs that remain promising candidates for forensic body fluid identification. The targeting of the significantly more stable miRNAs provides the potential to circumvent the challenge of analysing extensively degraded RNA. With our new research and careful adjustment of conventional RNA methodology to accommodate the specialised nature of forensic samples, we have overcome many of the difficulties associated with forensic RNA analysis. We have demonstrated that using MPS in a novel manner, we have improved current RNA detection methods and that MPS has the potential to become integrated into forensic casework for RNA body fluid/tissue identification.

35. TARGETED MULTIPLEXED NEXT GENERATION RNA SEQUENCING ASSAY FOR TISSUE SOURCE DETERMINATION OF FORENSIC SAMPLES

Hanson E.[1], Ingold S.[2], Haas C.[2], Ballantyne J.[1,3]

[1] *National Center for Forensic Science, University of Central Florida, Orlando, USA*
[2] *Institute of Legal Medicine, University of Zurich, Zurich, Switzerland*
[3] *Department of Chemistry, University of Central Florida, Orlando, USA*

Jack.Ballantyne@ucf.edu

The recovery of a DNA profile from the perpetrator or victim in criminal investigations can provide valuable 'source level' information for investigators. However, a DNA profile does not reveal the circumstances by which biological material was transferred. Some contextual 'activity level' information can be obtained by a determination of the tissue or fluid source

of origin of the biological material as it indicates some behavioral activity on behalf of the individual that resulted in its transfer from the body. Here, we sought to improve upon established molecular based methods for body fluid identification (m/miRNA profiling) by developing a targeted multiplexed next generation sequencing assay. The multiplexed biomarker panel includes several highly specific gene targets for each body fluid or tissue with the necessary specificity to definitevely identify all forensically relevant biological fluids and tissues (blood, semen, saliva, vaginal secretions, menstrual blood and skin). We demonstrate the sensitivity of the assay and the ability to identify body fluids in single source and admixed stains. The NGS-RNA body fluid identification panel not only provides 'activity level' information for criminal investigations, but will serve as the basis for a future assay which will also permit an association of a DNA profile to a specific body fluid or tissue through the analysis of coding region SNPs within each individual mRNA target. Thus use of this RNA-NGS system may provide forensic scientists with the routine capability of providing some activity level context to a specific individual's DNA profile.

36. ANALYSIS OF TISSUE SPECIFIC METHYLATION IN FORENSICS: THE PROBLEM OF BISULFITE CONVERSION

Holtkötter H.[1], Schürenkamp M.[1], Sibbing U.[1], Pfeiffer H.[1], Dennany L.[2], Tobe S.[3], Vennemann M.[1]

[1] *Institute of Legal Medicine, University Clinic Münster, Germany*
[2] *Centre for Forensic Science, University of Strathclyde, Glasgow, UK*
[3] *School of Biological Sciences, Flinders University, Adelaide, Australia*

Marielle.Vennemann@ukmuenster.de

Methylation of cytosines is an epigenetic modification that plays an essential role in various cellular processes such as gene regulation, cell development, and X-chromosome inactivation. In forensic science, methylation patterns are believed to be promising biomarkers for tissue type identification (1), as well as for age or sex determination (2, 3). The gold standard for the detection of DNA methylation is bisulfite genomic sequencing. Through e.g. qPCR it is possible to quantitatively determine the methylation status. However, it is well known that bisulfite conversion efficiency can vary massively (4). A non-optimal conversion results in overestimation of the methylation status. In this study, we investigated conversion efficiencies and present a positive control for bisulfite conversion in form of an artificial single-stranded DNA and two detectors to be included in each qPCR run. The DNA strand contains un-methylated cytosines that convert into thymines if bisulfite conversion is successful, and remain cytosines if unsuccessful. The first detector is labeled with the fluorophore FAM and contains a sequence complementary to cytosine, emitting green light if activated, while the second detector, containing a sequence complementary to thymine, is labeled with the fluorophore HEX, emitting yellow light if activated. Initial results of this study as well as bisulfite conversion efficiencies of various kits will be presented and the relevance of normalizing conversion efficiencies in forensic specimen discussed.

1. Park J-L, Kwon O-H, Kim JH, Yoo H-S, Lee H-C, Woo K-M, Kim S-Y, Lee S-H and Kim YS: Identification of body fluid-specific DNA methylation markers for use in forensic science. Forensic Science International. 2014, Volume: 13, Pages 147-15
2. Horvath S: DNA methylation age of human tissues and cell types. Genome Biology, 2013, Volume: 14, Pages R115
3. Piferre F: Epigenetics of sex determination and gonadogenesis. Developmental Dynamics, 2013, Volume: 242, Pages 360-370
4. Sasaki M, Anast J, Bassett W, Kawakami T, Sakuragi N, Dahiya R: Bisulfite conversion-specific and methylation-specific PCR: a sensitive technique for accurate evaluation of CpG methylation. Biochemical and Biophysical Research Communications, 2013, Volume: 309, Pages 305-309

37. MITOCHONDRIAL PORTRAIT OF SLAVIC – SPEAKING POPULATIONS AS REVEALED BY COMPLETE GENOME ANALYSES

Grzybowski T.

Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Poland

Recent developments in massively parallel sequencing (MPS) technologies have certainly sped up complete mitochondrial DNA analyses undertaken for both population genetic and forensic purposes. Nevertheless, complete mitogenome data from Slavic-speaking populations of Central and Eastern Europe are still relatively scarce. In this presentation, all available results from our research into mitogenome sequences from three major language groups which consist of western (Poles, Czechs, Slovaks), eastern (Russians, Belarusians, Ukrainians) and southern (Serbians, Croats, Slovenians) Slavs will be discussed on the basis of comparable data available from other populations of both Western and Eastern Eurasian ancestry. Special emphasis will be placed on the origin and molecular dating of subhaplogroups of potential Central- and Eastern European regional specificity within the major European clades of H and U. These results of mitogenome analyses will be interpreted in light of the data on the formation of a Slavic identity emerging from other genetic studies, as well as other disciplines, including archaeology, linguistics, history and physical anthropology.

38. COMPREHENSIVE ANALYSIS OF GENETIC VARIATION OF FORENSICALLY RELEVANT STR MARKERS IN SLAVIC POPULATIONS

Rębała K.

Department of Forensic Medicine, Medical University of Gdansk, Gdansk, Poland

k.rebala@gumed.edu.pl

Statistically significant differences in allele/haplotype distributions oblige the use of local databases whenever possible to estimate frequencies of genetic profiles and paternity index values in forensic casework. Contrary to autosomal STR loci regarded as sufficiently homogeneous for the purpose of forensic DNA typing in Europe, sex chromosomal markers display noticeable geographic differentiation within the continent. In the present study, variation of forensically relevant STR markers located on autosomes as well as X and Y chromosomes was compared in linguistically closely related Slavic-speaking populations inhabiting mainly Central, Eastern and Southeastern Europe. The dataset comprised 5043 individuals genotyped at 15 autosomal STR markers, 8106 individuals genotyped at 8 Y-STR markers and 3262 individuals (1723 males and 1539 females) genotyped at 4 X-STR markers. The results were referred to data on geographic and/or linguistic relations between the populations and on their demographic history. No geographically and/or linguistically driven genetic differentiation was observed for autosomal and X-chromosomal loci except for statistically significant departure of small Slavic communities isolated from their homeland populations. Scrutiny of Slavic Y-chromosomal STR haplotypes generally confirmed the previously detected genetic differentiation between populations speaking Northern and Southern Slavic languages. Implications of the observed genetic structure on statistical interpretation of forensic genetic evidence are presented and discussed.

39. EXAMPLES OF GENETIC IDENTIFICATION OF VICTIMS OF TOTALITARIAN REGIMES IN POLAND

Kuś M.[1], Ossowski A.[1], Zielińska G.[1], Kupiec T.[2]

[1] Department of Forensic Genetics, Pomeranian Medical University in Szczecin, Poland
[2] Institute of Forensic Research in Krakow, Poland

marta.kus1@gmail.com

Forensic genetics is one of the fastest growing field of science. New molecular technologies open a lot of new doors. Ten or fifteen years ago extracting DNA from a bone was hard to manage. Now, scientist are able to obtain a

full genetic profile even from an ancient bone. All the research and scientific advance impact the human identification process. Progress done by genetics has influence not only on criminal cases done by forensic labs. It gives also an opportunity to identify human remains which are exhumed after several dozen years.

Poland is a country which during the Second World War underwent a cruelty of two regimes. Trauma of one of them, namely communism, kept on going also years after the war ended. All the event which happened during this almost 20 years, caused thousands of nameless victims.

Need to give back the victims their names forced Polish scientist to start a project called Polish Genetic Database of Totalitarianisms Victims. Research started in December 2012, just after a first huge exhumation in Warsaw. After three years and numbers of field work done across the country, almost 500 human remains were found, among which more than 40 are identified.

My speech presents several identifications done by our team and shows the limitations which appeared during the process. The biggest problem was collecting a proper reference material. Second was obtaining proper DNA profiles from a bones which mostly come from a mass graves.

40. BEYOND BALDING-NICHOLS: INFERRING SUBPOPULATIONS IN A FORENSIC DNA DATABASE USING A LATENT VARIABLE APPROACH

Kruijver M.[1], Slooten K.[1,2], Meester R.[1]

[1] Department of Mathematics, VU University, Amsterdam, The Netherlands
[2] Netherlands Forensic Institute, The Hague, The Netherlands

m.v.kruijver@vu.nl

Forensic DNA databases can be modeled with a population genetic model. Typically, the Balding-Nichols model is applied with allele frequencies estimated from a population survey. A small positive theta-parameter is used to accommodate substructure within the surveyed population. This approach need not capture the heterogeneous structure of offender databases, which typically consist of groups of persons with various ethnic origins with unknown sizes. We propose an alternative approach, based on a latent variable model, which explicitly takes into account the existence of a small number of subpopulations in the database. The allele frequencies and the relative sizes of the subpopulations can be inferred from the offender database using the expectation maximization (EM) algorithm. This approach does not rely on possibly small and unrepresentative population surveys, but is driven by the actual genetic composition of the database only. We fit the model to a snapshot of the Dutch offender database (2014), which contains close to 180,000 profiles, and find that three subpopulations suffice to describe a large fraction of the heterogeneity in the database. The latent variable model has several applications. For instance, it can be used to improve estimates of true and false positive rates of database searches including familial searching and searches for a contributor to a complex mixture. Other potential applications include more efficient search strategies and estimation of the evidential value of a database match.

41. IMPLEMENTING AND VALIDATING INTERPRETATION SOFTWARE IN FORENSIC DNA CASEWORK: THE LRMIX STUDIO EXPERIENCE

de Jong J.[1], Gill P.[2,3], Haned H.[1]

presented by Benschop C.

[1] Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands
[2] National Institute of Public Health, Department of Forensic Biology, P.O. Box 4404 Nydalen, 0403 Oslo, Norway
[3] University of Oslo, Oslo, Norway

h.haned@nfi.minvenj.nl

Recently, a number of computer programs have been developed which are anchored in a likelihood ratio approach. These programs have greatly facilitated the interpretation of low-template mixed DNA profiles and their

reporting in court. In this presentation, we will discuss the development, validation and implementation of one such program, LRmix Studio, at the Netherlands Forensic Institute. LRmix Studio is a free, open-source software that implements a probabilistic model which explicitly accommodates the uncertainty in complex DNA profiles i.e. mixtures that are subject to the allelic drop-out and drop-in phenomena. The model is described by [1]. The program estimates these quantities from the available DNA profiles, and uses those estimates to generate likelihood ratios. LRmix Studio enables the evaluation of the probative value of low-template profiles that would have otherwise not been interpreted or reported in court.

LRmix Studio was introduced into casework and is routinely used in more than 30 forensic laboratories worldwide. In this presentation, we will highlight the methodology used for the validation and the implementation of LRmix Studio for forensic casework. We will also present the extensive testing of the performance of the underlying probabilistic model. Finally, we will discuss the challenges encountered and the next steps to improve and develop the existing software.

1. H. Haned et al. Exploratory data analysis for the interpretation of low template DNA mixtures. Forensic Sci. Int. Genet. 2012; 6(6); 762-774.

42. USING HD TRUE TESTS TO INFORM ON MODEL PERFORMANCE AND ADDRESS ADVENTITIOUS MATCHING

Taylor D.[1,2], John Buckleton[3], Evett I.[4]

[1] *Forensic Science South Australia, 21 Divett Place, Adelaide, Australia*
[2] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[3] *ESR, Private Bag 92021, Auckland 1142, New Zealand*
[4] *Principal Forensic Services Ltd, London, UK*

Duncan.Taylor@sa.gov.au

In 1950 Irving Good stated (quoting Turing) that "the expected factor for a wrong hypothesis in virtue of any experiment is 1". In likelihood ratio (LR) calculations using DNA profiling evidence this translates to "The average LR for a non-contributor of DNA will be 1". premise, however it seems still be to largely unrealised (and often surprising) in the forensic and legal community. This presentation explains the theory and how it arises, as well as the implications it has for the more familiar topic of adventitious matching. We suggest how the theory can be used to provide a simplistic statement to courts to explain how the LR relates to occurances of adventitious matches. Finally we demonstrate how this lemma can be used as a diagnostic for the performance of an interpretation systems and the effect that different interpretation systems have on the diagnostic.A number of commentators have published work utilising this.

43. EUROFORMIX: AN OPEN SOURCE SOFTWARE BASED ON A CONTINUOUS MODEL FOR EVALUATING COMPLEX STR DNA PROFILES WITH ARTEFACTS

Bleka Ø.[1,2], Gill P.[1,2]

[1] *Department of Forensic Genetics, Norwegian Institute of Public Health, Oslo, Norway*
[2] *University of Oslo, Oslo, Norway*

Oyvind.Bleka@fhi.no

We have released a freely available open source software named EuroForMix to analyze STR-DNA profiles in a user-friendly graphical user interface. The software implements a model to explain the allelic peak height from the PCR-process in order to do weight-of-evidence calculations. Through the unknown parameters in the model we are able to do inference on mixture proportion, the peak height properties, stutter proportion and degradation for any number of unknown and known contributors. In addition, EuroForMix takes care of allele drop-out, allele drop-in, coancestry effects and replicated samples. EuroForMix supports two inference approaches for likelihood ratio calculations. The first approach uses maximum likelihood estimation of the unknown parameters. The second approach is Bayesian based and integrates

out the unknown parameters by specify prior distribution for these. We illustrate the functionalities of the software and introduce guidelines to carry out the interpretation by considering a complicated real case example.

44. CONTINUOUS, FAST, AND THOROUGH MIXTURE ANALYSIS

Brenner C.[1,2]

[1] DNA·VIEW, Oakland, California, USA
[2] UC Berkeley Human Rights Center, Berkeley, California, USA

c@dna-view.com

A winning approach to mixture analysis must be as complicated as necessary but should be as simple as possible. Part of the art of modeling is to choose the model with an eye to priorities and consequences. Realities include dropout, ambiguity which alleles are present, stutter, masking of alleles, drop-in, and signal intensity variation, complications any reasonably adequate model must incorporate. An artful modeling simplification is to treat them as aspects of a single phenomenon – stochastic variation. Experimentation and development of the DNA·VIEW Mixture Solution seems to justify this approach.

A streamlined design has multiple benefits. One is speed – a user convenience of course, but not only that. Speed enhances software development by permitting more thorough testing and validation. Speed opens up a new and important vista: automated exploring. Thanks to enhanced detection sensitivity, mixture cases are ever more common and typically more complicated – including often multiple potential donor references. Comparing all combinations of the references plus any number of unknowns may mean a thousand possibly relevant inter-hypothesis likelihood ratios. Sorting through them manually and intuitively to judge which few are worth computing is next to impossible. Faced with such cases and given the gratifying speed of the Mixture Solution an obvious solution presented itself: a user interface that conveniently offers to compute them all. That works well and the term "mixture problem" thus has a bit of a new slant.

45. INTERPRETING MIXED DNA PROFILES CONSIDERING A RANGE IN THE ASSIGNED NUMBER OF CONTRIBUTORS

Cooper S. J.[1], McGovern C. E.[1], Abarno D.[2], Bright J. A.[1], Taylor D.[2,3], Buckleton J. S.[1,4]

[1] ESR, Private Bag 92021, Auckland 1142, New Zealand
[2] Forensic Science South Australia, 21 Divett Place, Adelaide, SA 5000, Australia
[3] School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, Australia
[4] NIST, Gaithersburg, Maryland, USA

catherine.mcgovern@esr.cri.nz

Recently, a number of fully continuous probabilistic methods have been described that model allelic and stutter peaks within a DNA profile. Such models can help address the known shortcomings of traditional methods of profile interpretation, such as those within a binary model. Nevertheless, even with such models there remains a level of subjectivity in interpretation of mixed DNA profiles, notably if there is a requirement to assign a likely number of contributors [1]. Within this study we use a continuous probabilistic software, STRmix™, to examine a number of mixtures where the ground truth number of contributors is known to determine whether updates to the software [2] will allow a meaningful interpretation of the mixtures without having to assign a specific number of contributors. Known single source two and three person mixtures were created by computer simulation and also in vitro; amplified using ABI's GlobalFiler™ multiplex. The results were analysed proposing a range in the likely number of contributors, where the ground truth (*N*) plus and minus one contributor was considered. The results were compiled and demonstrated that the use of probabilistic software can facilitate the interpretation of complex mixed DNA profiles where an analyst considers a range in the likely number of contributors. We discuss how this may help address areas of uncertainty in interpretation, extend the number of samples where a statistic can be applied and compel a level of concordance between different analysts.

1. Cooper SJ, McGovern CE, Bright JA, Taylor D and Buckleton JS: Investigating a common approach to DNA profile interpretation using probabilistic software. Forensic Science International: Genetics. 2015; 16:121 – 131
2. Taylor D, Bright JA and Buckleton JS: Interpreting forensic DNA profiling evidence without specifying the number of contributors. Forensic Science International: Genetics. 2014; 13: 269 – 280.

46. DISTINGUISHING MIXTURE DONORS FROM THEIR RELATIVES

Slooten K.[1, 2]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*
[2] *VU University, Amsterdam, The Netherlands*

k.slooten@nfi.minvenj.nl

Likelihood ratio (LR) calculations on DNA mixtures are nowadays able to deal with allelic dropout and drop-in. This allows to obtain LR's for mixtures where this was not previously possible, but it also means that - in order to be a possible contributor - a full match with the crime stain is no longer necessary. If the LR opposes the suspect to an unrelated person, then the false positive probability is bounded in case the suspect is actually unrelated to the mixture donors, whether or not artefacts are possible. However, no such bound applies to relatives: in that case both hypotheses (suspect is a mixture donor or is unrelated to them) are incorrect. Relatives of a donor typically share more alleles with the mixture than unrelated individuals, and we investigate what the inclusion risks are when they are subjected to a standard LR calculation. We show how to calculate the risks that such a LR exceeds a chosen threshold, and that false inclusions are indeed more likely than for unrelated persons. We investigate various types of mixtures, and compare the obtained risks to those for mixtures without artefacts. Calculations are done with a binary approach, where the LR's are based on the observed alleles and the dropout probabilities for each donor. These models are widespread in the forensic community. We study the importance of having accurate estimates of each donor's dropout probability. Finally we emphasize the need for LR calculations that incorporate relatedness.

47. DNA AND RNA PROFILING OF HUMAN CELL MATERIAL RESIDING ON PUBLIC AND PRIVATE OBJECTS AND AFTER ACTIVITY SCENARIO'S

van den Berge M.[1], Clarisse L.[1], Keetels A.[1], Lindenbergh A.[1], Ozcanhan G.[1], Zijlstra S.[1], Sijen T.[1]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*

m.van.den.berge@nfi.minvenj.nl

Along with the increased sensitivity of DNA profiling systems comes the increased possibility of genotyping trace amounts of background cell material. To gain our understanding on the amount of DNA, number of contributors and in addition the cell types that prevail in surfaces that contribute to background traces, 105 public objects (*e.g.* bank notes, stair rails) and 323 private objects (*e.g.* jeans, washed items and hands) were analysed using DNA and RNA profiling. STR results for the majority of samples showed multiple contributors. While for most public objects no major contributor could be deduced, this was possible for most private samples. Besides, DNA transfer was assessed on 142 'activity' samples which included dragging and grabbing experiments performed on mainly the clothed areas of individuals. Deduced majors did not always correspond to the 'perpetrator' in the activity scenario; sometimes this contributor could not be distinguished from background signals on the dragged object. Additionally, we see that high DNA yields do not necessarily relate to an increased number of contributors or specific cell types. Skin was the cell type predominantly observed in all samples, with the occasional detection of saliva. Overall, results of this study increase our understanding regarding the prevelance of human cell material in background and activity scenarios.

48. EXPLORING THE RELATIVE DNA CONTRIBUTION OF FIRST AND SECOND OBJECT'S USERS ON MOCK TOUCH DNA MIXTURES

Oldoni F.[1], Castella V.[1], Hall D.[1]

[1] *Forensic Genetics Unit, University Hospital of Lausanne, Lausanne, Switzerland*

fabio.oldoni@unil.ch

Contact stains recovered at burglary/robbery crime scenes frequently result in mixtures of major and minor DNA from the first (owner) and second user of an object, respectively. Understanding the relative contribution of DNA left behind by different users overtime will lead crime investigators to more robustly evaluate the likelihood of detecting an individual's profile based on the type and history of an object (1). To address such issue a contact stain simulation-based protocol was designed.

Over 250 touch DNA mixtures from both porous (P) and non-porous (NP) items, commonly found at break-in crime scenes were simulated. A cohort of 14 volunteers was chosen to act either as first or second item's user. The object's owner was required to handle/wear regularly P and NP items over an 8-10-day period, whereas the second user for 5`, 30` and 120` (minutes), in three different simulation sessions.

Our results showed a linearly increased profile percentage contribution of the second user compared to the first. Moreover, the contribution from second users increased up to 22% and 42% upon 5` use and then to 41% and 72% after 120` for P and NP objects, respectively, suggesting substrate-dependent DNA deposition. Alleles of unknown source were detected at 2% and ≤10% of the total profile for P and NP objects.

Interestingly, when considering NP items handled for 120 minutes, the second user became the major DNA contributor. The observation of unexpected alleles suggested the occurrence of secondary DNA transfer. A more comprehensive analysis is ongoing.

1. van Oorschot RA, Glavich G, Mitchell RJ: Persistence of DNA deposited by the original user on objects after subsequent use by a second person. Forensic science international Genetics. 2014; 8(1): 219-25.

49. A MOLECULAR EXPLORATION OF HUMAN DNA/RNA CO-EXTRACTED FROM THE PALMAR SURFACE OF THE HANDS AND FINGERS

Lacerenza D.[1], Omedei M.[2], Aneli S.[1], Gino S.[1], Pasino S.[1], Berchialla P.[3], Robino C.[1]

[1] *Department of Public Health Sciences and Pediatrics, University of Turin, Turin, Italy*
[2] *Department of Public Health, University of Rome "Tor Vergata", Rome, Italy*
[3] *Department of Clinical and Biological Sciences, University of Turin, Turin, Italy*

carlo.robino@unito.it

„Touch DNA" refers to DNA that is left behind when a person touches or comes into contact with an item. The source of touch DNA, however, is still debated, and the large variability in DNA yield from casework samples suggests that different body fluids, other than skin, can be transferred through contact.

To better understand the nature and characteristics of touch DNA, samples were collected from the palmar surface of the hands and fingers of 30 male and 30 female donors by tape lift/swabbing, and subjected to DNA/RNA co-extraction. It was shown by multiplex mRNA profiling that cellular material other than skin was present on 33.3% of the male and 18.3% of the female hands. The average DNA yield from these samples (0,70 ng/µl) was significantly higher compared to samples containing only skin cells (0,08 ng/µl) ($p=0.012$).

The integrity of the DNA isolated from the donors' hands and the prevalence of DNA mixtures was evaluated by STR typing, in comparison with reference STR profiles from buccal swabs. DNA profiles containing, on average, 79.6% of the donors' alleles were obtained from samples including cellular material other than skin, compared to 66.5% in samples with just skin. The average

percentage of foreign alleles was 18.1% in females and 7.6% in male profiles. The occurrence of mixed DNA profiles appeared, therefore, significantly dependent on donors' gender ($p<0.001$) rather than on tissue source ($p=0.637$).

Finally, no significant difference in DNA source, integrity and prevalence of DNA mixtures was found between dominant and non-dominant hand.

50. ASSESSING PRIMARY, SECONDARY AND TERTIARY DNA TRANSFER USING THE PROMEGA ESI-17 FAST PCR CHEMISTRY

Davies C.[1], Thomson J.[2]

[1] Risley casework site, LGC, United Kingdom
[2] LGC, Teddington, United Kingdom

Craig.Davies@LGCGroup.com

The current generation of PCR chemistries used in many forensic science laboratories offer improved sensitivity and detection. In a criminal justice setting, it is as important as ever that the scientist has the means to be able to evaluate the issue of how and when DNA might have been deposited [1].

We have used the Promega ESI-17 Fast system to investgiate DNA transfer in a series of three experiments. The first experiment is designed to measure typical levels of DNA transfer when a person handles an object (primary transfer). The second and third introduce hand shaking prior to handling the object to measure and compare typical levels of DNA transfer as a result of secondary and tertiary transfer events, respectively.

This study compares the completeness (peak count) and magnitude (peak height) of DNA profiles produced as a result of each of these types of transfer, as well as assessing the contribution from non-donor DNA accumulated from the workplace surroundings. Repeated experiments have been conducted using a set of 16 staff participants with limited allele sharing and differing propensities for DNA transfer.

The reults show clear examples of primary and secondary transfer, including examples where the degree of secondary transfer exceeds primary transfer in the same sample. Unambiguous tertiary transfer was difficult to detect but cannot be ruled out. We will present a summary of the findings and with case examples will outline how this extensive bank of data can assist scientists in offering more robust evaluations of the significance of casework findings.

51. THE DEPOSITION AND PERSISTENCE OF INDIRECTLY-TRANSFERRED DNA ON REGULARLY-USED KNIVES

Meakin G. E.[1,2], Butcher E. V.[1,3], van Oorschot R. A. H.[4], Morgan R. M.[1,2]

[1] Centre for the Forensic Sciences, University College London, London, UK
[2] Department of Security and Crime Science, University College London, London, UK
[3] Division of Biosciences, University College London, London, UK
[4] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia

Considerations of how DNA recovered from a crime scene got there are of increasing importance to forensic casework[1]. While the possibility of indirect DNA transfer is well established, research into such transfer is limited to the handling of DNA-free items. This study therefore investigated whether secondarily-transferred DNA can be detected on regularly-used items, and if so, for how long might it persist. Volunteers used knives regularly, such that DNA amounts recovered from the handles (1-10 ng) were comparable to those previously reported for regularly-used items[1]. Each regular user then shook hands with another volunteer ('handshaker') and repeatedly stabbed one of their regularly-used knives into foam for 60 seconds. DNA was recovered from knife handles using mini-tapes at approximately one hour, one day, and one week after the stabbings. In three of the four pairings of volunteers, complete and partial DNA profiles matching those of the regular user and handshaker respectively, at ratios of ~1:10, were recovered from the knives within one hour. Alleles attributed to the handshaker were still detected after one week,

but were significantly reduced in number for two pairings. Unknown alleles were also recovered from the knives, suggesting other indirect DNA transfer events; e.g. alleles attributed to the DNA profile of a volunteer's partner were repeatedly detected. For the fourth pairing, only complete single-source DNA profiles matching the regular user's profile were recovered. This study demonstrates that, on regularly-used items, secondarily-transferred DNA can be detected and can persist for at least a week; this has implications for forensic reconstructions.

1. Meakin G and Jamieson A: DNA transfer: Review and implications for casework. Forensic Science International: Genetics. 2013; 7: 434 – 443.

52. STABBING AND DNA TRANSFER

Samie L.[1], Hicks Champod T.[1], Taroni F.[1], Castella V.[2]

[1] School of Criminal Justice, University of Lausanne, Switzerland
[2] Forensic Genetics Unit, Faculty of Biology and Medicine of Lausanne, Switzerland

Lydie.Samie-Foucart@unil.ch

It is now possible to analyze very minute quantities of DNA. This has many advantages, but the drawback of this technological progress is that interpretation of the results becomes increasingly complex: mixtures are more common than they used to be and major complications arise (i.e., drop-in and drop-out phenomena).The possibility of secondary and tertiary transfer is now becoming an issue.

The purpose of this research was twofold: first to study the transfer of DNA from the handler and secondly to observe if handlers would secondarily transfer DNA from relatives or colleagues. We choose to mimic cases where the offender would attack a person with a knife and the suspect has nothing to do with the incident.

The transfer experiments showed that the profile of the person handling the knife was most of the time present as the major profile. However, in some case, it only partially matched, the handler becoming the minor contributor at some of the loci. No traces allowed excluding the handler, however precautions were taken to minimize background DNA. When results were not interpretable, we considered that the handler's DNA was absent. DNA profiles of the offender's relatives/colleagues were not observed either.

It is hoped that this research will help forensic scientists assign the probability of their DNA results given prosecution's proposition that the person stabbed the victim. This research also provides data for assessing the absence of a profile. More research will be needed to assign the probability of the results given an alternative activity.

53. RESULTS OF THE GHEP-ISFG COLLABORATIVE EXERCISE FOR THE TAXONOMIC IDENTIFICATION OF FORENSIC SAMPLES USING THE SPINDEL METHOD

Alves C.[1,2], Pereira R.[1,2], Prieto L.[3], Amorim A.[1,2,4], Pereira F.[5] on behalf of the Spanish and Portuguese Speaking Working Group of the ISFG (GHEP-ISFG)

[1] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[2] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal
[3] Grupo de medicina Xenómica. Instituto de Ciencias Forenses. Universidade de Santiago de Compostela, Spain
[4] Faculty of Sciences, University of Porto, Porto, Portugal
[5] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Porto, Portugal

calves@ipatimup.pt

The SPInDel multiplex PCR allows the identification of species by the generation of numeric profiles combining the lengths of six mitochondrial ribosomal RNA (rRNA) gene regions[1,2]. Here we describe a collaborative exercise for the taxonomic identification of forensic samples using the SPInDel kit carried out in 2014 by the GHEP-ISFG. A total of 24 laboratories from 10 countries were supplied with a SPInDel primer mix and control samples of the 10 target species needed to perform genotyping of 11 samples from previous

GHEP-ISFG Intercomparison Exercises. Overall, correct identifications were reported by 22 of the 24 laboratories. The errors were concentrated in a few laboratories, with one laboratory reporting errors in all profiles. The success rate in the identification of species with the SPInDel kit was 100% in 8 of the 11 samples. The level of concordance in identifications was always higher than 93%, including in samples with low amounts of DNA (hair shafts) and mixtures of saliva and blood. When considering all cases together, 98.6% of the reported profiles yielded correct species identifications. The frequency of wrong (5.8%) and missing (1.9%) alleles was low and did not interfere with the correct species identification, mainly because the SPInDel method relies on the analysis of multiple loci. In summary, the SPInDel method was easily implemented by different laboratories and genotyping platforms and the interpretation of results was straightforward. The method proved to be efficient in the identification of species in diverse forensic samples.

1. Pereira F, Carneiro J, Matthiesen R, van Asch B, Pinto N, Gusmão L and Amorim A: Identification of species by multiplex analysis of variable-length sequences. Nucleic acids research. 2010; 38: e203-e203.
2. Carneiro J, Pereira F and Amorim, A: SPInDel: a multifunctional workbench for species identification using insertion/deletion variants. Molecular ecology resources. 2012; 12: 1190-1195.

54. SPECIES IDENTIFICATION USING HIGH RESOLUTION MELTING (HRM) ANALYSIS WITH RANDOM FOREST CLASSIFICATION

Bowman S.[1], Peters L.[1], Roffey P.[2], Richardson A.[3], McNevin D.[1], Gahan M.[1]

[1] National Centre for Forensic Studies, University of Canberra, Bruce ACT, Australia
[2] Forensics, Australian Federal Police, Canberra ACT, Australia
[3] Faculty of Education, Science, Technology and Mathematics, University of Canberra, Bruce ACT, Australia

sorelle.bowman@canberra.edu.au

Species identification and differentiation is an important aspect of forensic investigations and represents a viable means to link biological sources of evidence, either human or non-human in origin, to multiple victims, offenders and crime scenes. This can be achieved rapidly with high resolution melt (HRM) analysis targeting the universal 16S rRNA gene.

Human and ten non-human species commonly encountered as either consumed meats (e.g. cow), domestic pets (e.g. dog) or location specific road kill (e.g. kangaroo) were assayed. Parameters assessed included variation within and between HRM plates and species and ability to identify and discriminate between species. Results showed that traditional derivative melt curves, obtained using onboard ViiA™ 7 Real Time PCR System software (Life Technologies), lacked the discriminating power required for consistent species classification.

Employing a variety of random forest algorithms (such as classification trees, bagging and boosting) to the randomly partitioned training set provided highly sensitive and specific classification for the holdout test set. The results from the proposed random forest classification algorithm have significantly outperformed the onboard ViiA™ 7 Real Time PCR System software for both non-human and human species classification.

55. NEW APPROACHES IN FORENSIC ENTOMOLOGY NGS TECHNOLOGY FOR THE IDENTIFICATION OF PUPAL AGE RELATED MOLECULAR MARKERS

Zajac B. K.[1], Amendt J.[1], Horres R.[2], Verhoff M. A.[1], Zehner R.[1]

[1] Institute of Legal Medicine, Forensic Biology, University Hospital, Goethe University, Frankfurt, Germany
[2] GenXPro GmbH, Altenhöferallee 2, 60438 Frankfurt am Main, Germany

zajac@med.uni-frankfurt.de

Blow flies belong to necrophagous insects and are attracted by corpses which usually serve as oviposition site already shortly after death. Decaying

corpses provide an optimal food source/ecology/habitat for the development of juvenile blow flies. The key task of forensic entomology is the estimation of the minimal post-mortem interval (PMImin) using the knowledge about the development and biology of necrophagous insects. The development and growth rate of juvenile blow flies is dependent on temperature and species. Considering this, age of blow fly larvae can be estimated by comparing the larval length and weight to species specific development data. While PMImin estimation is well established for blow fly larvae current studies focus on aging blow fly pupae. Among all discussed methods gene expression analysis is predominant. It has been discussed that too high variance between biological replicates is challenging to assign a specific value of gene expression to a certain development stage. To manage this challenge we analyzed NGS generated transcriptomes of 15 different development stages of Calliphora vicina pupae. Massive Analysis of cDNA Ends (MACE) provided high resolution gene expression data, which was used to identify new transcripts up-regulated for only a certain day during pupal development [1]. In total 53.939 distinct transcripts have been tested. Based on the MACE data qPCR assays for each day of pupal development have been designed and tested regarding their specificity and efficiency. We present the first steps towards the development of an age estimation kit for Calliphora vicina pupae.

1. Zajac BK, Amendt J, Horres R, Verhoff MA, Zehner R: De novo transcriptome analysis and highly sensitive digital gene expression profiling of Calliphora vicina (Diptera: Calliphoridae) pupae using MACE (Massive Analysis of cDNA Ends). Forensic science international. Genetics. 2015; 15:137–146

56. ROBUST AND REPRODUCIBLE DNA TYPING OF SOILS

Khodakova A.[1], Burgoyne L.[1], Abarno D.[1,2], Linacre A.[1]

[1] School of Biological Sciences, Flinders University, Adelaide, Australia
[2] Forensic Science South Australia, Adelaide, Australia

adrian.linacre@flinders.edu.au

Soil is encountered commonly in forensic investigations yet rarely forms part of the examination process. This is due to a lack of a robust and reliable DNA typing method where the resulting data from suspect, victim, crime scene, reference material or alibi samples can be compared. An additional issue is that soil samples adhering to items submitted for analysis can be small therefore limiting the testing available. We have developed[1] a method that provided reproducible metagenomic datasets from multiple samples taken from the same site throughout the year. These datasets have been found to be different to data obtained from soils of similar appearance but separated by 3 km and different again from soil types that were visually different. Twenty one soil samples were collected from three sites at four time points through the year. The whole biota of the soil sample as small as 50 mg was targeted in the proposed approach highlighting the advantages of DNA typing based on High-Throughput DNA Sequencing. The application of metagenomic software such as QIIME and MG-RAST along with the reference microbial databases allowed for three distinct clusters of microbial assemblages to be formed according to the samples' collection sites. Based on the obtained data likelihood models can be developed and reliable conclusions be drawn that in turn directly results in potential application to forensic practice.

1. Khodakova A, Smith RJ, Burgoyne L, Abarno D, and Linacre A: Random Whole Metagenomic Sequencing for Forensic Discrimination of Soils. PLoS ONE. 2014; 9(8) e104996

57. REVIEWING SCIENTIFIC LITERATURE

Butler J. M.

National Institute of Standards and Technology, Gaithersburg, Maryland, USA john.butler@nist.gov

Science benefits from effective communication of ideas. Research results are shared with others through publications and presentations. Scientific publication involves efforts in reading, writing, and reviewing the literature

[1]. Editors of peer-reviewed journals rely on input from scientific colleagues to judge the merits of submitted manuscripts. Knowledgeable reviewers providing timely feedback are important for a successful peer-review process. This presentation will share insights based upon editorial experience with *Forensic Science International: Genetics* as well as extensive writing practice in preparing five textbooks and over 150 research articles and invited book chapters. Reviewing manuscripts is a chance to provide an important service and to influence the scientific community for good.

In addition to discussing approaches to reading, writing, and reviewing relevant literature, some recent articles covering forensic genetics will be considered. By reviewing where the forensic genetics community is focusing efforts in the scientific literature, we may be able to better chart the future of the field [2].

1. Butler, J. M. (2013) The triad of scientific publication: reading, writing, and reviewing. Forensic Sci. Int. Genet.: Suppl. Ser. 4(1): e115-e116.
2. Butler, J. M. (2015) The future of forensic DNA analysis. Phil. Trans. R. Soc. B 370: 20140252; DOI: 10.1098/rstb.2014.0252.

58. GHEP COLLABORATIVE SIMULATED EXERCISE FOR DVI / MPI: LESSONS LEARNED ON LARGE SCALE PROFILE DATABASE COMPARISONS

Vullo C.[1], Romero M.[1], Catelli L.[1], Saragoni V. G.[2], Jiménez Pleguezuelos M. J.[3], Romanini C.[1], João Anjos Porto M.[4], Puente Prieto J.[5], Bofarull A.[6], Hernández A.[7], Farfán M. J.[8], Prieto V.[9], Álvarez D.[10], Penacino G.[11], Zabalza S.[12], Bolaños A. H.[13], Manterola I. M.[14], Prieto L.[15], Parsons T.[16]

[1] Equipo Argentino de Antropología Forense (EAAF), Córdoba, Argentina
[2] Unidad de Genetica Forense, SML, Santiago de Chile, Chile
[3] Unitat Central de Laboratori Biològic, Divisió de Policía Científica, Mossos d'Esquadra, BCN, España
[4] Instituto Nacional de Medicina Legal e Ciências Forenses, I.P, Coimbra, Portugal
[5] LabGenetics, Madrid, España
[6] Instituto Nacional de Toxicología y Ciencias Forenses (INTCF), Barcelona, España
[7] Instituto Nacional de Toxicología y Ciencias Forenses, (INTCF), Canarias, España
[8] Instituto Nacional de Toxicología y Ciencias Forenses, (INTCF), Madrid, España
[9] Instituto Nacional de Toxicología y Ciencias Forenses, (INTCF), Sevilla, España
[10] Laboratorio de ADN, Comisaría General de Policía Científica, Madrid, España
[11] Unidad de Análisis de ADN (COFyBCF), CABA, Argentina
[12] Unidad de Laboratorio NASERTIC & Policia Foral, Navarra, España
[13] Análisis ADN Forense, Depto. Ciencias Forenses, Poder Judicial, Costa Rica
[14] Servicio de Genómica - SGIker – Universidad del País Vasco (UPV/EHU), España
[15] Instituto de Ciencias Forenses, Grupo de Medicina Xenómica, Universidade de Santiago de Compostela, España
[16] International Commission for Missing Persons (ICMP), USA

cvullo@yahoo.com.ar

The GHEP-ISFG Working Group has recognized the importance of assisting DNA laboratories to gain expertise in handling DVI or missing persons identification (MPI) projects which involve the need for large scale genetic profile comparisons. Laboratories were invited to participate in a DNA matching exercise on a simulated autosomal STR database representing unidentified victims and family reference profiles. The database was comprised of 87 hypothetical profiles from unidentified victims, with the possibility of commingling, and 286 family reference profiles representing diverse pedigrees. Eleven laboratories participated in the exercise, with the goal to correctly discover re-associations and family matches. The results of direct matching for commingled remains re-association were correct and fully concordant among all laboratories. However, the kinship analysis for missing persons identifications showed variable results among the participants. There was a group of laboratories with fully correct, coincident results but nearly half of the others showed discrepant results. Three main errors were detected: a) some laboratories did not use the complete reference family genetic data to report the match with the remains, b) the prosecutor and/or defense hypotheses were sometimes wrongly expressed for the likelihood ratio calculations, and c) many laboratories did not properly evaluate the prior odds for the event. The results suggest that large scale profile comparisons

for DVI or MPI is a challenge for the Forensic Genetics laboratories that are not used to dealing with such events; statistical treatment of a match and the Bayesian framework should be also better standardized into the group.

1. Scientific Working Group on DNA Analysis Methods (SWGDAM). Guidelines for Missing Persons Casework. http://www.swgdam.org/
2. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, Scheithauer g R, Schmitter H, Schneider PM; DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI); Forensic Science International: Genetics 1 (2007) 3–12.
3. Sozer A, Baird M, Beckwith M, Harmon B, Lee D, Riley G, Schmitt S; Guidelines for Mass Fatality DNA Identification Operations, AABB; http://www.aabb.org/programs/disasterresponse/Documents/aabbdnamassfatalityguidelines.pdf

59. QUALITY EXERCISE IN DISASTER VICTIM IDENTIFICATION

Immel U. D.[1], Lutz-Bonengel S.[2], Lessig R.[1]

[1] Institute of Legal Medicine of the Martin-Luther-University of Halle-Wittenberg, Germany
[2] Institute of Legal Medicine of the Albert-Ludwigs-University of Freiburg, Germany on behalf of the participating laboratories

Following the Interpol standard is one of the primary methods to identify unknown persons the forensic molecular analysis of samples taken from the victims. In many cases of the routine case work these persons were found in a stadium of high decomposition after a long time. Following the recommendations of the ISFG bone samples have to investigate.

Quality exercises are part of the quality control in several parts of the routine case work since many years. For example the GEDNAP in forensic stain analysis or several societies of paternity testing established such controls which are part of the accreditation of a laboratory too. In mass disaster cases or cases of identification such quality exercises are not established so far. The working group inside the German working party of the ISFG decided to establish a quality exercise using samples of the routine case work. Femurs collected from full body donors of the Institute of Anatomy were stored under defined conditions for 12 weeks in 2014. 34 laboratories from Europe took part at this test and got a bone sample. The results of this quality exercise will be reported.

60. FACTORS INFLUENCING THE RELIABILITY OF THE BONE SAMPLE DNA TYPING RESULTS

Vanek D.[1, 2], Budowle B.[3, 4], Dubska J.[1]

[1] Forensic DNA Service, Prague, Czech Republic
[2] Charles University in Prague, 2nd Faculty of Medicine, Prague, Czech Republic
[3] Institute of Applied Genetics, University of North Texas Health Science Center, TX 76107, USA
[4] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

The quality and reliability of the DNA typing results produced by research and forensic laboratories are limited by the amount and condition of the samples processed and influenced by the applied laboratory practice(s). Because of the often limited quantity and quality of DNA in bone samples, even low level cross-contamination can become a serious problem for obtaining reliable results. The results of the Collaborative exercise organized by the Forensic DNA Service and the Institute of the Legal Medicine, 2nd Faculty of Medicine, Charles University in Prague, Czech Republic in collaboration with several other institutes has shown that concordant results can be obtained from a common sample among laboratories that use varied extraction procedures, different commercial STR kits, in-house mtDNA protocols, and laboratory specific interpretation guidelines. The chance of false negative or false positive identification results is increasing with the longer post-mortem interval and also due to the different environmental factors and common

laboratory errors. The presenting author will provide the auditorium the list of recommendations for the more reliable DNA typing of bone samples.

61. IMPLEMENTATION OF THE EXPANDED CODIS CORE LOCI IN THE UNITED STATES

Hares D.

FBI Laboratory, Quantico, Virginia, United States

Douglas.Hares@ic.fbi.gov

In March of 2015, the Federal Bureau of Investigation (FBI) published the expansion of the original CODIS core 13 loci to a new CODIS core containing 20 loci with an implementation date of January 2017 for U.S Laboratories[1]. The selection was based on a consortium validation effort of 11 CODIS laboratories and National Institute of Standards and Technology (NIST) using a variety of instrumentation. With the National DNA Index System (NDIS) approaching 15 million DNA profiles, data on specific operational issues regarding database searching strategies will be discussed. NDIS has always required forensic profiles to contain 10 of the 13 core loci for routine searching at the national level. As NDIS has continued to grow in size, searches of partial profiles and mixtures containing common alleles have become problematic. The presentation will explore a new concept for searching forensic profiles nationally. The concept utilizes a forensic profile's estimated match rarity to determine the searching suitability. It is anticipated that the new searching concept will be used as part of the implementation of the new CODIS 20 core loci.

1. Hares D: Selection and Implementation of Expanded CODIS Core Loci in the Unites States. Forensic Sci. Int. Genet. (2015); http://dx.doi.org/10.1016/j.fsigen.2015.03.006 page 33 – page 34

62. ANALYSIS OF UNI AND BI-PARENTAL MARKERS OF MIXTURE SAMPLES: LESSONS FROM THE 22ND GHEP-ISFG INTERCOMPARISON EXERCISE

Toscanini U.[1,5], Gusmão L.[2,3,4], Salas A.[5,6], Fernández K.[7] on behalf of the Spanish and Portuguese-Speaking Working Group of the ISFG (GHEP-ISFG)

[1] PRICAI-Fundación Favaloro, Buenos Aires, Argentina
[2] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Brazil
[3] IPATIMUP (Institute of Pathology and Molecular Immunology from de University of Porto), Portugal
[4] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[5] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain
[6] Diseases and Vaccines Unit, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain
[7] National Institute of Toxicology and Forensic Sciences (INTCF), Ministry of Justice, Madrid, Spain

secretaria@ghep-isfg.org; utoscanini@pricai.com.ar

Since 1992, the Spanish and Portuguese-Speaking Working Group of the ISFG (GHEP-ISFG) has been organizing annual Intercomparison Exercises coordinated by the INTCF from Madrid, aiming to provide proficiency tests for forensic DNA laboratories. Each annual exercise comprises a Basic (recently accredited under ISO/IEC 17043:2010) and an Advanced Level, both including a kinship and a forensic module. Here, we present the results for autosomal and sex chromosomes STRs, and mtDNA in two forensic samples "M4" (Basic level, mixture 2:1 (v/v) saliva/blood) and "M8" (Advanced level, mixture 4:1 (v/v) saliva/semen) from the 2014 GHEP-ISFG Intercomparison Exercise. Discrepancies other than typos or nomenclature errors (over the total allele calls) represented 6.5% ("M4") and 4.7% ("M8") for autosomal STRs, 10.1% ("M4") and 7.8% ("M8") for X-STRs, while the lowest error rates were observed for Y-STRs: 1.3% ("M4") and 0.0% ("M8"). Different number of laboratories participated and reported results (13 to 72) for each sample

and type of markers. Drop-out and drop-in alleles appeared as the main cause of errors, with different criteria used to include minor peaks and stutters. Common commercial kits showed different performance for autosomal STRs. Also, the analysis of electropherograms revealed that the proportions of the contributors detected in the mixtures varied among the participants. There was no agreement for the mtDNA results of M4: while some laboratories documented a single control region haplotype, others reported unexpected profiles (suggesting contamination problems). There were additional issues concerning nomenclature of heteroplasmies. For M8, most laboratories detected only the haplotype corresponding to the saliva.

63. INTER AND INTRA-VARIATION OBSERVED FROM A NIST INTERLABORATORY STUDY ON DNA MIXTURE INTERPRETATION IN THE U.S. (MIX13)

Coble M. D.[1], Bright J. A.[2], Curran J.[3], Butler J. M.[1]

[1] National Institute of Standards and Technology, Gaithersburg, MD, United States
[2] The Institute of Environmental Science and Research, Auckland, New Zealand
[3] The University of Auckland, Auckland, New Zealand

mcoble@nist.gov

Complex DNA mixtures from more than two individuals and/or profiles with small quantities of DNA, can be challenging for the analyst to interpret. Most laboratories have developed their profile interpretation protocols based on internal validation studies, publications in the scientific literature, training workshops, and guidance from scientific groups such as SWGDAM and the DNA Commissions of the ISFG.

Interlaboratory studies allow a "big picture" view across different laboratories and can be used to measure consistency among analysts using the same protocol within the same laboratory. Since 1997, NIST has conducted a number of DNA mixture interlaboratory studies for the forensic community. In 2005 Butler and Kline [1] conducted an interlaboratory study (MIX05) with electronic data that showed a wide amount of variation between and within the 69 participating laboratories.

After the publication of the 2010 SWGDAM Autosomal STR Interpretation Guidelines [2], many laboratories in the U.S. established analytical and stochastic thresholds for their mixture interpretation protocols. The MIX13 interlaboratory study was designed to determine the "lay of the land" within the U.S. in regards to STR mixture interpretation, subsequent to the publication of those guidelines. Examples of the mixture interlaboratory study from the 108 participating laboratories will be presented. Ideas for future training and research to improve mixture interpretation and reporting will be discussed.

1. Butler JM and Kline MC (2005) „NIST Mixture Interpretation Interlaboratory Study 2005 (MIX05)" poster at the 16th International Symposium on Human Identification (Grapevine, TX), September 27-28, 2005.
2. SWGDAM (2010) Autosomal STR Interpretation Guidelines: http://www.swgdam.org/Interpretation_Guidelines_January_2010.pdf

POSTER ABSTRACTS

Poster Listing by Theme

No.	Presenting Author	Title
	Theme 01	**Population Genetics**
1	Tikumporn Thooptianrat	Genetic similarity scale of tandem repeat variability in Thai human genome
2	Toshimichi Yamamoto	Potential statistical differentiation between Japanese and Korean populations with about 100 STRs
3	Miguel Marino	Forensic and population genetic analyses of 20 STRs loci in Mendoza population
5	Daniel Corach	Analysis of the genetic structure of Santa Cruz province and it comparison with the other Southern Patagonian provinces of Argentina
6	Luiz A. Ferreira Da Silva	Male ancestry of afro derived populations of Alagoas, Brazil, determined by analysis of 24 Y chromosome SNPs
7	Yahya Khubrani	Population genetic data of 21 STR markers in Saudi population
8	Leonor Gusmao	Analysis of admixture in Native American populations from Colombia
9	Antonio Salas	The Near Eastern component in present-day Tuscans from the 1000 Genome Project
10	Antonio Salas	The genomic legacy of the Transatlantic Slave Trade in the Yungas Valley of Bolivia
11	Antonio Salas	Mapping the genomic mosaic of two 'Afro-Bolivians' from Yungas (Bolivia)
12	Antonio Salas	Genetics provides new clues on the ethno-history of the Maya and the 'ladinos' from Guatemala
13	Antonio Salas	Ancient DNA analysis reveals origins of 17th-century enslaved Africans
14	Antonio Salas	New genomic insights into the westernmost edge of the European Romani diaspora
15	Juan Jose Builes	Ancestral genetic composition in a population of South Western Colombian using autosomal AIM-InDels
16	Beatriz Martinez	Ancestry background of a population sample from Bolivar Department, Colombia
17	Beatriz Martinez	InDels markers set and ancestry estimates in a population sample from Atlantic Department of Colombia

18	Lena Poulsen	Results of 16 autosomal STRs studied in population samples from Turkey, Iraq, Lithuania, Slovenia and Albania
19	Yiping Hou	Genetic polymorphism of 22 autosomal STR loci in Chinese Han population
20	Maria Joao Porto	Usefulness of PowerPlex fusion system as typing methodology of CODIS and ESS genetic markers in a immigrant population from Cabo Verde in Lisboa
21	Antonio Amorim	Autosomal indels distribution in metropolitan Manila, Philippines
22	Tatiana Hessab	F values and adjusted LR in Brazilian populations
23	Rashed Alghafri	Population data for SE33 locus in United Arab Emirates Arab population
24	Rashed Alghafri	An investigation of 21 insertion deletion markers in United Arab Emirates population
25	Anibal Gaviria	Analysis of the most efficient autosomal STRs and genetic data for the locus SE33 in Ecuadorian population
26	Anibal Gaviria	Allelic frequencies and forensic parameters for MiniSTRs D10S1248, D14S1434 and D22S1045 (NC01) in a sample from Central Andean Colombian region
27	Barbora Emmerova	Missing population studies on new European Standard set loci (ESS) and its impact on population statistics
28	Maria Eugenia DAmato	Evaluation of DIPplex Investigator in European, Asian and African populations
29	Peter Ristow	Evaluation of AmpFLSTR Identifiler Loci within South African and Botswana Populations
30	Eida Almohammed	Development and Validation of an allelic Frequency Database for Qatari population using 13 Rapidly Mutating Y-STRs Multiplex Assay

	Theme 02	**X-Chromosome Variation**
31	Juan Jose Builes	X-STRs as a tool for missing persons identification using only siblings as reference
32	Yan Liu	X-SNP typing of degraded DNA from formalin-fixed tissues by MALDI-TOF MS
33	Mario Scherer	Development and validation of the new Investigator Argus X-12 QS Kit
34	Susana Jimenez	Development of a new 17 X-STRs multiplex for a higher genetic resolution in forensic and anthropological studies
35	Joanna Wysocka	Allele and haplotype frequencies of 12 X-STR loci of Investigator Argus X-12 Kit in a population of northern Poland
36	Sergey R. Borovko	Belarusian experience of the use of FamLinkX for solving complex kinship cases involving X-STR markers
37	Lena Poulsen	Results of 12 X-chromosomal STR markers studied in populations from Turkey, Iraq, Lithuania and Slovenia
38	Jeanett Edelmann	Paternity testing of two female siblings with Investigator Argus X-12 Kit: a case with several rare mutation and recombination events

39	Anibal Gaviria	Genetic data of 10 X-chromosome STR loci in Ecuadorian population
40	Juan Jose Builes	Genetic data of 10 X-STR in an Afro-descendant population sample of the Department of Chocó - Colombia
41	Daniel Corach	Analysis of 33 X-InDel panel in Argentinean population
42	Antonia Picornell	Genetic diversity of 12 X-chromosomal short tandem repeats in Jewish populations
43	Miguel Bolea	X-chromosomal haplotype frequencies of four linkage groups in a population of Argentina
44	Peter M Schneider	Allele nomenclature implications of new hidden sequence variants at DXS10148, DXS10074 and DXS10134 loci

	Theme 03	Ethical and Legal Issues
45	Petra Maaskant-van Wijk	From sub-source to source: interpreting results of biological trace investigations using probabilistic models
46	Charlotte Word	What does this statement really mean?
47	Bas Kokshoorn	Beyond the source: evaluating DNA evidence at activity level in casework
48	Vladislav Zavarin	Exclusion rates in court-directed and personal information paternity tests in Russian Federation
49	Zsolt Padar	Misinterpretation of sample contamination in a Hungarian casework
50	Bas Kokshoorn	DNActivity: international cooperation in activity level interpretation of forensic DNA evidence

	Theme 04	Lineage Markers
51	Kiyoshi Minaguchi	Construction and application of multi-plex PCR for mtDNA sequencing from highly degraded and small amounts of DNA
52	Siddhita Gopinath	Developmental validation of the Yfiler plus PCR amplification Kit
53	Martin Bodner	Helena's many daughters: massively parallel sequencing provides further insights into the most common west eurasian mtDNA control region haplotype
55	So Yeun Kwon	Investigation into the Y-STR typing using next generation sequencing
56	Monika Stoljarova	Massively parallel sequencing of multiplex short amplicons of mtDNA for analysis of challenged forensic samples
57	Li Li	Results of typing of 71 biallelic markers on Y chromosome in Xinjiang Han population of China
58	Petra Kralj	Ancient DNA analysis to uncover migrations in medieval Trondheim
59	Natalie Weiler	Development of a control region based mtDNA snapshot assay
60	Fang-Chin Wu	The polymorphisms of 23 DNA Y-STR loci in Taiwan residents

61	Antoni Asensio	Ibermitobase: a search tool for mtDNA population data in the Iberian Peninsula
62	Samuel T. G. Ferreira	23 Y-STR database of samples from sexual assaults in Brazil
63	Aqeela Abuidrees	Rapid amplification of 13 rapidly mutating Y-STRs in a multiplex PCR assay
64	Anna Barbaro	Validation of PowerPlex Y23 system (Promega) using reduced reaction volume
65	Weibo Liang	Development of an octuplex ChrY miniSTR system
66	Rashed Alghafri	An evaluation of rapidly mutating Y-STR multi-allelic markers
67	Rashed Alghafri	Comparison between Yfiler and RM Y-STRs in United Arab Emirates population
69	Joanna Chamier-Ciemińska	Presence of Y-chromosome sequences in DNA of young female victim of sexual assault
70	Zoltan Szabolcsi	Mitochondrial DNA analysis for prediction of relatedness among victims of Second World War
71	Monica Omedei	mtDNA role in mixtures deconvolution
72	Nano Nagle	Improved resolution of mitochondrial macrohaplogroup m in Aboriginal Australians; evidence for a novel, and ancient M42 haplogroup
73	Catarina Xavier	New insights into the Arawakan diaspora: mtDNA control region analysis of two populations
74	Julianne Henry	The benefits and limitations of expanded Y-STR loci
75	Daniel Corach	Analysis of high mutable Y-STRs in urban and Amerindian Argentinean populations
76	Maria Mercedes Lojo	The finding of Y-STR microdeletion involving DYS448, DYS392, DYS549 and DY385a/b markers in a paternity case with deceased alleged father
77	Anibal Gaviria	Y STRs mutation events in father-son pairs in Ecuadorian individuals
78	Sohee Cho	Genetic analysis of Y-chromosomal STR of six populations in Asia
79	Kyoung-Jin Shin	Massively parallel sequencing of mitochondrial DNA control region and SNPs for global haplogroup determination
80	Mikkel Meyer Andersen	Choosing analytical thresholds for Yfiler Plus dyes
81	Venusia Cortellini	Evaluation of mtDNA stability across the maternal line: a study on three generations
82	Elizeu Carvalho	Mitochondrial DNA direct PCR sequence of blood FTA paper
83	Elizeu Carvalho	Characterization of the STR loci allele's distribution of Y chromosome with high mutation rate in population sample of Rio de Janeiro
84	Mannis van Oven	PhyloTree Build 17: growing the human mtDNA tree

85	Conrado Martinez-Cadenas	Cosegregation analyses of Y chromosomes and Spanish surnames reveal features of interest to police investigations
86	Lu Zhang	Mitochondrial DNA typing of laser-captured single sperm cells to differentiate individuals in a mixed seminal stain
87	Zheng Wang	Differentiating between monozygotic twins in forensics through Next Generation mtGenome sequencing
88	Katsuya Honda	The mitochondrial DNA polymorphisms in chromosomal aberration detected by NGS
89	Christina Strobl	Full mt-Genome sequencing with mitochondrial tiling path primers using the Ion Torrent PGM
90	Judith Zander	Proof-of-principle-study for the investigation of short mitochondrial DNA fragments using Next Generation Sequencing
91	Yingnan Bian	Population genetics of 26 Y-STR loci in the ethnic minority from Fujian Province, Southeast China
92	Antonio Salas	Regional patterns of Y-chromosome variation in Bolivia
93	Antonio Salas	Examining pattern of mitochondrial DNA variation in Chile, from North to the most Southern cone
94	Antonio Salas	Analysis of Y chromosome haplotypes in Chile: a comprehensive national study
95	Antonio Salas	The extinct mitochondrial DNA clade retrieved from a 500-years-old South American Inca mummy
96	Leonor Gusmao	Detecting the paternal genetic diversity in West Africa using Y-STRs and Y-SNPs
97	Antonio Salas	Mosaic maternal ancestry in the Great Lakes region of East Africa
98	Evelyn Guevara	Assessing the genetic diversity in the extant Chachapoya population from northeastern Peru using uniparental DNA markers
99	Harald Niederstätter	Of Alps and men: on social and Y chromosomal markers from Tyrol, Austria
100	Miguel Marino	Asymmetries in maternal and paternal genetic contribution in Mendoza population and the role of Africans
101	Berardi G., Llull C.	Exploring regional patterns of Y-chromosome variation in Argentina
102	Regina M. Barretto Cicarelli	Mitochondrial DNA: 30 SNPs are enough to discriminate an admixed population from Espirito Santo state, Brazil
103	Jung Ho Hwang	Population genetic data and concordance study for the PowerPlex Y23 system and the AmpFlSTR Yfiler Kit in Koreans
104	Anne-Marie Pflugbeil	Genotyping of 52 Y-chromosomal haplogroup specific SNPs for skeletal remains from an excavation find from the Migration Period
105	Maria Joao Porto	Y-SNPs genetic variation in the Caucasian population living in south Portugal
106	Maria Joao Porto	Analysis of Y chromosomal STRs in Southern of Portugal population with PowerPlex Y23
107	Maria Joao Porto	Study of Y-SNPs genetic markers with forensic interest and ancestry informative power in PALOPs immigrant populations in Lisboa - preliminary results
108	Sheng-Ping Hu	Genetic link between Chaoshanese and other Chinese populations: evidence from mitochondrial DNA

109	Joana F Ferragut	Founding mothers of Chueta population
110	Adriana Castillo	Comparing different population groups in Santander - Colombia through Y-STR haplotype analysis
111	Luis Alvarez	Exploring sephardic lineages in Sao Tome e Principe
112	Leonor Gusmao	Y-STR haplotype background of Philippines: comparison with other Southeast Asian populations
113	Luis Alvarez	Enriching the knowledge on East Asia populations: characterization of male lineages from Macau and Shanghai
114	Leonor Gusmao	Genetic characterization of 27 Y-STR loci in the native population of Ashaninka from Peru
115	Atsushi Nagai	Genetic polymorphisms at 17 Y-STR loci in an Egyptian population
116	Marian M. de Pancorbo	Forensic interest of the dissection of the main european paternal lineage M269 in Atlantic Europe and Iberia
117	Naseem Malik	Resolution of Y haplotypes using the Life Technologies Yfiler Plus VTS PCR Amplilfication Kit

	Theme 05	Massively Parallel Sequencing
118	Christian Hussing	Comparison of techniques for quantification of next-generation sequencing libraries
119	Christopher Phillips	A catalog of collateral variants in and around core forensic STRs from NGS analysis of the HGDP-CEPH diversity panel
120	Ilaria Carboni	Evaluation of the reliability of the data generated by targeted next generation sequencing from artificially degraded DNA samples
121	Kyoung-Jin Shin	Massively parallel sequencing of global STR Loci in Koreans
122	SallyAnn Harbison	Massively parallel sequencing of Identifiler and Power Plex Y amplified forensic samples
123	Bruce Budowle	Chemistry and performance testing of forensically-relevant genetic marker systems within the Ion PGM System
124	Bhavik Mehta	An assessment of the Illumina MiSeq platform for forensic SNP analysis
126	Laurence Devesse	Concordance of massively parallel sequencing results using the illumina MiSeq FGx with current methods of STR typing
127	Jodi Irwin	Evaluation of massively parallel sequencing technologies for expanded DNA identification capabilities at the Federal Bureau of Investigation Laboratory
128	Weibo Liang	Forensic-Loci-finder (FLfinder): tool for analyzing forensic loci data of second generation sequencing
129	Christian Hussing	Test of the Illumina ForenSeq Kit
130	Stefano Caratti	MiSeq FGx sequencing system: a new platform for forensic genetics
131	Helle Smidt Mogensen	Template preparation of AmpliSeq libraries using the Ion Chef

132	Dennis McNevin	Allele frequency and coverage thresholds for forensic SNP genotyping with massively parallel sequencing
133	Anders Buchard	Next generation sequencing of reference samples with the HID-Ion Ampliseq Identity Panel using single punches from FTA-cards
134	Catarina Xavier	High-throughput sequencing of forensic STRs and SNPs using the MiSeq benchtop sequencer
135	Joseph Chang	Recovering genomic and whole mitochondrial profiles from single hair roots with qPCR and next generation sequencing
136	Ernie Guzman	Development and validation of a targeted next generation sequencing solution for forensic genomics
137	Jon Davoren	Development of a DNA mixture deconvolution solution for forensic evidence using massively parallel sequencing
138	Weibo Liang	The study of a panel consisting of STRs, SNPs and indels on Illumina Mi-Seq platform
139	Carolina G Bottino	STR genotyping with Ion Torrent PGM and STR 10-plex system: highlights on performance and data interpretation
140	Suhua Zhang	Human identification of 120 SNP with PGM Sequencer System
141	Suhua Zhang	Pilot study of 124 SNP included in HID-Ion ampliseq panel in a four-generation family
142	Henrik Green	Genotyping 27 bones using Illumina ForenSeq DNA Signature Kit
143	Ugo Ricci	Forensic genetics in NGS era: new frontiers for massively parallel typing
144	Soren Vilsen	Modelling noise in Second Generation Sequencing forensic genetics STR data using a one-inflated (zero-truncated) negative binomial model
145	Katherine Gettings	Sequence-based analysis of stutter at STR loci: characterization and utility
146	Marian M. de Pancorbo	Next generation sequencing as an alternative method of cloning PCR products for ancient DNA authentication

	Theme 06	**New Technologies**
148	Erica Romsos	Rapid DNA maturity assessment
149	Sudhir Sinha	Retrotransposable elements: novel and sensitive DNA markers and their application in human identity
150	Francesca Fontana	DEPArray digital sorting of biological mixtures achieves isolation of 100%-pure cell populations for clear-cut genetic analysis
151	Frederic Grosjean	A new approach for the separation of spermatozoa from other cell types in forensically relevant samples
152	Allison Holt	New Y-Screen assay using the Quantifiler Trio DNA Quantification Kit
153	David King	Validation of a rapid DNA process using a platform optimized for booking station environments
154	Christophe Frippiat	Anhydrobiosis as an alternative to freezing for the preservation of extracted DNA. Comparison of Qiasafe and DNAStable LD
155	Weibo Liang	Microhaplotype: a potential genetic marker to supplement the standard CODIS panel

156	Natalia Oliveira	DNA biosensor as a confirmatory test: studies of household cleaners effects of onto electrochemical DNA detection
157	Tetsushi Kitayama	Evaluation of the quality of STR profiles generated by modified rapid DNA systems
158	Maria Joao Porto	Validation of Quantifiler Trio DNA Quantification Kit in forensic samples
159	Maria Joao Porto	Quantifiler Trio DNA validation and usefulness in casework samples
160	Marielle Vennemann	Linear-after-the-exponential (LATE)-PCR: improved asssymetric PCR for quantitative DNA-analysis

	Theme 07	Predictive DNA Analysis
161	Maria de la Puente	The G-AIMs nano SNP set: a 31-plex SNaPshot assay genotyping many of the most informative forensic aims
162	Amke Caliebe	The prediction of pigmentation phenotypes in the Northern German population
163	Mayra Eduardoff	Practical evaluation of SNP-based prediction of male pattern baldness in a sample of convicted felons
165	Ramani Anantharaman	Ancestry prediction in South East Asian populations using the Illumina ForenSeq Kit on the MiSeq FGx
166	Clemence Hollard	On the use of the HID-Ion AmpliSeq Ancestry Panel in a forensic case
167	Runa Daniel	Forensic validation of the HID-Ion AmpliSeq Ancestry Panel on the Ion Torrent PGM
168	Diana Hall	Potential use of DIP-STR as ancestry informative markers based on the CEPH human genome diversity panel survey
169	Chris Syn	Age prediction with dual-Taqman probe sjTREC quantification assay
170	Ana Freire-Aradas	Interpretation of male pattern baldness-associated DNA variants using the Snipper online classifier
171	Ewelina Pośpiech	Comparison of different mathematical methods for DNA-based eye colour predicton applicable in forensics
172	Magdalena Marcińska	Frequency differences of SNP alleles associated with male pattern baldness in Africa, Asia and Europe
173	Leonor Gusmao	Assessing the suitability of different sets of InDels in ancestry estimation
174	Vania Pereira	Typing of 111 ancestry informative markers in an Albanian population
175	Ozlem Bulbul	Studies of East European populations with a 46-plex ancestry-informative InDel set
176	Ozlem Bulbul	Inferring Southwest Asian Ancestry with an efficient panel of SNPs
177	Eugenio Alladio	An alternative method for the evaluation of the ethnic origin of unknown genetic profiles
178	Christopher Phillips	Population studies analysing variation in a 46-plex AIM-Indel forensic ancestry assay
179	Simona Severini	Use of ancestry-informative markers as a scientific tool to combat the illegal traffic in human kidneys
180	Carme Barrot	Correlation between the molecular basis of impulsivity and suicidal behavior

181	Titia Sijen	Invader assays for rapid analysis of phenotypic traits

	Theme 08	DNA Typing Methods
182	Arisa Sanubol	Genetic markers for sex identification of Thais in forensic DNA analysis
183	Weibo Liang	A novel system for forensic SNP analysis through PCR- Ligase Detection Reaction
184	Mario Scherer	Improving sample analysis and interpretation using Qiagens latest Investigator STR Multiplex PCR assays with a novel Quality Sensor
185	Diana Hall	Early non-invasive prenatal paternity test based on DIP-STR analysis
186	Rashed Alghafri	An evaluation of miniSTR markers in casework samples
187	Bobby LaRue	An evaluation of a new Rapid DNA platform for field forward applications
188	Dennis McNevin	Direct-to-PCR tissue preservation for DNA profiling
189	David Moore	A review of single source live case work samples with optimal DNA inputs processed with the PowerPlex ESI17 Fast Kit
191	Haibo Luo	Development of a SNP-STRs multiplex for forensic identification
192	Kevin Kiesler	A strategy for the characterization of single nucleotide polymorphisms in a reference material
193	Manuel Fondevila Alvarez	Non-binary SNPs revisited: a new forensic snapshot assay of 29 multiple-allele SNPs
194	Masaaki Hara	Effects of storage method on DNA degradation in old bloodstain samples
195	Lina Norén	DTT quenches the passive reference signal in Real-Time PCR
197	Peter Gunn	The reproducibility, accuracy & reliability of low copy number DNA profiling using multiple sampling of profiles
198	Helle Smidt Mogensen	Test of ESSplex_SE_QS with quality sensor
199	Corina Benschop	Enhanced interrogation for low template DNA samples
200	Malin Sanga	A panel of PCR-inhibitory reference materials for quality evaluation of multiplex STR analysis kits
201	Shanan Tobe	Evaluation of direct PCR in cases of alleged sexual assault
202	Gabriella Mason-Buck	Concordance study of four DNA amplification chemistries
203	Anne-Marie Pflugbeil	Initial experience in the application of the PowerQuant system and the ESSplex SE QS Kit for ancient DNA analysis
204	Mario Scherer	Analysis and interpretation of difficult samples - the interaction of a suitable DNA quantification kit with the right STR assay may facilitate processing of critical trace material

205	Paolo Fattorini	Systematic study on the analytical parameters relevant to achieve reliable STR profiles, as assessed in a multicentre data set
206	Danilo Braganholi	Insertion of amelogenin marker in the 38 HID-INDELs multiplex reaction
207	Edna Iwamura	DNA from stains of semen and seminal fluid pre and postvasectomy recovered after 10 years of storage
208	Natalia Oliveira	DNA genotyping analysis overview: profile of rape cases in the state of Pernambuco, Brazil
209	Anibal Gaviria	Easy and fast procedure to isolate, purify and immortalize DNA fragments for allelic ladders construction
211	Camilo Vilela Lamego	On the use of the DNAscan Rapid DNA Analysis System (GE Healthcare)
212	Vladimir Orekhov	Pre-amplification as a tool for resolving mixed DNA profiles
213	Sasitaran Iyavoo	Reduced PCR cycling time amplification using AmpFℓSTR Identifiler Kit
214	Sasitaran Iyavoo	Reduced volume PCR amplification using AmpFℓSTR Identifiler Kit
215	Cintia Fridman	Is it possible to use forensic DNA phenotyping in Brazilian population?
216	Bushra Idris	Evaluation of the Chelex-100 method and four commerical DNA extraction systems
217	Alicia Haines	Successful Direct STR amplification of anagen hair follicles after nuclear staining
218	Alicia Haines	Duration of in situ fluorescent signals within anagen hairs
219	Zlatko Jakovski	Comparative study of two DNA extraction methods in different tissues and conditions of degradation
220	Adrian Linacre	The end of bad hair days
221	Zhonghui Thong	Evaluation of the RapidHIT 200 System: a comparative study of its performance with Maxwell DNA IQ/Identifiler plus/ABI 3500xL workflow
222	Lais Vicente Baptista	Rebalancing of degraded DNA profiles through preferential extraction
223	Anna-Liina Rahikainen	Evaluation of DNA extracted from up to 16 years old post-mortem blood FTA cards using Quantifiler Human Plus (HP) Quantification Kit
224	Susi Pelotti	Analysis of aged seminal stains by current forensic DNA approach
225	Michael Josef Schwerer	Comparison of DNA preparation results from autopsy tissue specimens with or without previous postmortal CT angiography
226	Kirstin Janssen	Validation of automated PCR set-up of Quantifiler Trio DNA Quantification Kit on the Biomek 4000 laboratory automation workstation
227	Kazuhiko Tsukada	Efficiency of DNA extraction from urine test strips
228	Maria Joao Porto	Direct amplification of reference samples with GlobalFiler PCR Amplification Kit

229	Maria Joao Porto	Direct amplification of casework samples in fabrics using GlobalFiler PCR Amplification Kit
230	Maria Joao Porto	Testing the behavior of GlobalFiler PCR Amplification Kit with degraded and/or inhibited biological samples
231	Maria Joao Porto	DNA quantification by Real-Time PCR in different forensic samples
232	Daniel Corach	Development and validation of a human DNA quantification and sex determination approach based on real time PCR followed by high resolution melting analysis
233	Yiping Hou	Validation of a multiplex system with 20 multi-Indels for forensic purposes
234	Venus Kallupurackal	Automating PrepFiler Forensic DNA Extraction Kit: optimization and validation on Freedom EVO 150
235	Mounia Bessekri	Novel forensic DNA profiling metrics for a universal quantitative validation scheme
236	Peter Gunn	Investigation into ethylene oxide residuals on downstream DNA analysis
237	Nicoletta Cerri	Validation of the AmpFlSTR NGM SElect PCR Amplification Kit using ABI PRISM 310 Genetic Analyzer
238	Weibo Liang	A 21-plex DIP panel's application in multinational Chinese population
239	Yiping Hou	Screening of Multi-InDels markers on X-Chromosome for forensic purpose

	Theme 09	DNA Databases
240	Thomas Callaghan	Booking station DNA analysis and searching of CODIS databases
241	Carolyn Steffen	Consortium validation project data assessment to finalize the expanded CODIS core Loci in the U.S.
242	Samuel Ferreira	The use of DNA database of biological evidence from sexual assaults and rapes in criminal investigations: a successful experience in Brazil
243	Gabriele Savoldi	Performance testing of the semi-automated CPA200 and fully-automated CPA300 card processing instruments
244	Gian Carlo Iannacone	Algorythm aligen: simple method of encryption and matching with genetic profiles in a system of STR identification database
245	Sally-Ann Harbison	New Zealand's DNA profile databank- celebrating 20 years of success
246	Peter Gill	SmartRank: a likelihood ratio software for searching national DNA databases with complex DNA profiles

	Theme 10	Body Fluids Identification
247	Jacob de Zoete	A probabilistic approach for the interpretation of RNA profiles as cell type evidence
248	Margreet van den Berge	Advancing forensic RNA typing: non-target bodily secretions, a nasal mucosa marker, a differential extraction protocol and the

		relation between DNA and RNA profiling sensitivity
249	Graham Williams	Performing body fluid identification using a microRNA multiplex with capillary electrophoresis
250	Patricia Albani	Novel messenger RNAs for body fluid identification
251	Graham Williams	Body fluid specific microRNA markers: characterising the fluctuation of expression in vaginal material when analysed over a full menstrual cycle
252	Weibo Liang	MicroRNA markers for forensic body fluid identification obtained from miRCURY LNA Array
253	Weibo Liang	Influences of different qPCR methods on forensic body fluids identification by microRNA
254	Weibo Liang	Screening of microRNA markers for distinguishing between menstrual and peripheral blood
255	Eva Sauer	Validation of forensic body fluid identification based on empirically normalized micro-RNA expression data
256	Jessica Teschner	Tissue-specific-methylation pattern assay in casework
257	Hannah Holtkoetter	Validation of an immunochromatographic D-dimer test to presumptively identify menstrual fluid in forensic exhibits
258	Galina Kulstein	MicroRNA profiling for characterization of (micro) traces
259	Daniele Podini	Confirmatory detection of sperm via proximity ligation Real Time PCR
260	Weibo Liang	Comparison of three co-extraction kits for total RNA and genomic DNA from mouse cardiac muscle
261	Anna Barbaro	Evaluation study about the Seratec rapid tests
262	Gunn-Hege Olsen	Additional fixation improves the performance of SPERM HY-LITER Express
263	Pilar Martinez	Less bright CF488 fluorescence signals in non-spermatic nuclei
264	Christian Gausterer	Validation of tissue-specific DNA methylation markers for the differentiation between menstrual and peripheral blood
265	Miriam Sirker	Impact of using validated or standard reference genes for miRNA qPCR data normalization in cell type identification
266	Olatunde Afolabi	Reference genes study: a requisite in qRT-PCR experiments
267	Kevin Chong	Development of a RNA profiling assay for biological tissue and body fluid identification
268	Paolo Fattorini	Reliability of rt-PCR from RNA degraded samples: an in vitro model
269	Steffi Köcher	Searching for new methylation sites for tissue identification
271	Yiping Hou	Identification of body fluid using tissue-specific DNA methylation markers
272	Natasha Arora	What microbial species are suitable for body fluid identification? An in silico assessment using short read data
273	Kiwon Park	Development of rapid and on-site confirmatory saliva test by multiplex realtime PCR of three oral microorganisms

274	Youn-Hyoung Nam	Simple method for the identification of saliva and vaginal fluid by detecting Streptococcus species and Lactobacillus species
275	Rinaldo Jucker	In-house validation of an RNA/DNA co-extraction strategy for body fluid identification / STR profiling and application to forensic casework

	Theme 11	Touch DNA
276	Adrian Linacre	DNA profiles from fingermarks
277	Franz Neuhuber	Contamination in obtaining trace evidence an issue more topical than ever?
278	Pamela Tozzo	Effect of two different swabs on genetic profiling of enhaced fingerprints
279	Jana Joel	DNA extraction from forensic adhesive tape lifts - a comparison of two different methods
280	Andrea Gladys Colussi	DNA recovery from sloughed epithelial cells: a retrospective analysis
282	Ane Elida Fonnelop	Persistence and secondary transfer of DNA from previous users of equipment
283	Christina Forsberg	Reference material for comparison of different adhesive tapes for forensic DNA sampling
284	Duncan Taylor	Vectors of DNA transfer in a laboratory environment
285	Bianca Szkuta	Self versus non-self DNA in handprints
286	Gabriele Margiotta	Risk of DNA transfer by gloves in forensic casework.
287	Dayse Silva	Evaluation of collection and extraction methodologies of latent fingerprints for military application
288	Roland van Oorschot	Activities between activities of focus- relevant when assessing DNA transfer probabilities
289	Roland van Oorschot	Shedder status- reality or myth?
290	Bianca Szkuta	Residual DNA on examination tools following use
291	Celine Pfeifer	Persistence of trace DNA on burglary related objects

	Theme 12	DVI Human Remains
292	Arnoud Kal	DNA identification of the victims of Malaysia Airlines flight MH17 crash
293	Andrzej Ossowski	Polish Genetic Database of Totalitarianism Victims
294	Daniel Vanek	Impact of fixatives on quality and quantity of nucleic acids in archived formalin fixed tissues
295	Samuel T. G. Ferreira	A comparative study between muscle, cartilage and bone samples for DNA typing in disaster victim identification (DVI)

296	Denice Higgins	Postmortem degradation of teeth; implications for DNA identification of human remains
297	Jessica Rothe	Development of an evaluation-form for an improved data exchange in the field of DNA extraction from skeleton remains
298	Aikaterini Papaioannou	CMP Cyprus Genetics Unit: working with chalenging cases
299	Renata Jacewicz	Traces of Nazi Repression in Lodz region of Poland - genetic verification of historical truth
300	Carolina G Bottino	Urinary bladder swabs as a source of DNA for human identification
301	Sylvain Amory	Assessment of a simple postmortem DNA sampling and typing method for efficient DVI and missing persons identification
302	Monika Karija Vlahovic	The QuickGene System for isolation DNA from old skeletal samples
303	Andrea Gladys Colussi	Robust and efficient network for DNA recovery from human remains
304	Maria Mercedes Lojo	DNA recovery from a gallstone in an action for damages
305	Dragana Zgonjanin	Case report: DNA identification of burned skeletal remains
306	Dayse Silva	Optimized DNA extraction method from skeletal remains using different typing methodologies in forensics
307	Fulya Eylem Yediay	Comparison study for the commercial STR kits on ancient human samples
308	Jitka Votrubova	Pre-extraction removal of PCR inhibitory agent (vivianite) found on excavated bones
309	Dayse Silva	Bone DNA typing using a new miniYSTR multiplex
310	Samuel T. G. Ferreira	A comparative study between muscle, cartilage and swab from inside the urinary bladder samples for DNA typing of severely burnt bodies in disaster victim identification (DVI)
311	Grażyna Zielińska	Examples of exceptional human identifications done by Department of Forensic Genetics in Szczecin, Poland
312	Ugo Ricci	Genetic identification of burned corpses as a part of disaster victim identification effort
313	Dayse Silva	DNA extraction and purification from archival material: revisiting the standard operating procedure
314	Claudia Gomes	A maternity case with human remains from a XIII-XIV century burial at Uceda, Guadalajara, Central Spain
315	Maria Joao Anjos	Restored teeth can be used as samples for genotyping?
316	Stijn Desmyter	A combined DNA extraction method for bones and teeth
317	Weibo Liang	A primary investigation on SNPs associated with eyelid traits of Chinese Han adults
318	Kazuho Maeda	The case of 2 siblings that identified not only by DNA profiling
319	Marian M de Pancorbo	A grave in my garden. Genetic identification of Spanish Civil War victims
320	Claudia Gomes	Study of medieval critical samples - A genetic approach to the study of the Iberian Muslims

321	Kazuhiko Tsukada	DNA extraction from teeth left exposed to the elements for more than 60 years using silica membrane column
322	Claudia Gomes	Nondestructive extraction DNA method from bones or teeth, true or false?
323	Cemal Gurkan	Population genetics of Turkish Cypriots from Cyprus: forensic and anthropological implications
324	Maria Joao Porto	SNPforID 52plex in casework samples: "cracking" bones and other difficult samples
325	Daniel Vanek	The collaborative exercise concept on DNA typing of bone samples
326	Claudia Gomes	Looking for a reliable criteria for the establishment of solid STR profiles using ancient critical samples from 3000 to 4000 years ago
327	Claudia Gomes	Kinship analysis in mass graves: evaluation of the blind search tool of the Familias 3.0 software in critical samples

	Theme 13	Biostatistics
328	Sho Manabe	Development of new peak-height models for a continuous method of mixture interpretation
329	Corina Benschop	Beyond the limits of DNA typing
330	Guro Dorum	Linked markers in mixtures
331	Nadia Pinto	Theory and statistics of mutation rates: a mathematical framework reformulation for forensic applications
332	Andreas Tillmar	Ilir, a biostatistical tool for analysing the overall impact of genetic linkage for an arbitrary set of markers aimed at forensic identity and relationship testing
333	Frank Goetz	Forensic biostatistics with cloud computing
334	Daniel Russell	A simple unconstrained semi-continuous model for calculating likelihood ratios for complex DNA mixtures
335	Mikkel Meyer Andersen	Assessing evidential strength of a Y-chromosomal STR match
336	Peter Gill	The open-source software lRmix can be used to analyse SNP mixtures
337	Jiri Drabek	Activity level in the hierarchy of propositions in case of the People of the State of California v. Orenthal James Simpson analyzed using Bayesian network
338	Antonio Salas	Parametric approach to kinship testing
339	Yiping Hou	Comparison of two online algorithm methods for forensic ancestry inference
340	Ozlem Bulbul	Investigation of the mixture interpretation compatibility with validation and simulation studies
341	Alessandra Caglia	Is peak height important for the statistical evaluation of the weight of evidence in DNA mixtures?

342	Massimo Lancia	Complex DNA mixture analysis: report of two cases
343	Helen Burri	Accounting for linkage between the STR loci D5S818/CSF1PO and vWA/12S391 in kinship analyses: impact on likelihood ratio values
344	Peter M. Schneider	Drop-out model optimization on degraded DNA samples
345	Navreet Kaur	Open software for relationship inference based on mixtures
346	Eugenio Alladio	The dark side of DNA interpretation in complex cold cases: "The risk of a wrong identification when facing with LT-DNA mixtures"
347	Eugenio Alladio	An alternative application of the consensus method to DNA typing interpretation for LT-DNA mixtures
348	Charles Brenner	The DNA VIEW Mixture Solution
349	Tomonori Tamura	Effects of genetic linkage in sib test using the GlobalFiler Kit

	Theme 14	Paternity
350	Sergey Borovko	Mutation rates at 17 STR loci in the Belarussian population
351	Eriko Ochiai	Mutation of tetra-nucleotide STR in parent-child relation using GlobalFiler Kit
352	Eva-Maria Dauber	A genetic inconsistency at the D7S820 locus multi-step mutation, uniparental isodisomy or other?
353	Yiping Hou	The application of multi-InDel as supplementary in paternity cases with STR mutation
354	Melissa Ferrian	Sibling incest
355	Melissa Ferrian	Two mothers and two fathers for two half-brothers' pairs
358	Yiping Hou	Screening age-related CpGs for forensic purpose in Chinese Han population
359	Gustavo Adolfo Penacino	Heteropaternal superfecundation: implicancies in forensic genetics
360	Chie Morimoto	The effect of linkage on sibship determination based on likelihood ratio
361	Mario Gysi	Non-invasive prenatal paternity testing: a case report
362	Weibo Liang	Mutation study of 28 autosomal STR loci in Southwest Chinese Han population
363	Xudong Wang	An approach for exclusion of full-sibling relationship when multiple known full-siblings included-5th allele exclusion method

	Theme 15	Forensic Biology
364	Alicia Haines	Finding DNA: using fluorescent in situ detection

365	Melanie Grabmuller	On the effect of shot distance, ballistic model construction and doping and the type of gun on the simultaneous analysis of DNA and RNA from backspatter recovered from inside surfaces of firearms
366	Lay Hong Seah	Separation patterns of sperm mixtures: the major-minor impact
367	Michael Josef Schwerer	Extraction of DNA from gastric content in different stages of digestion
368	Daniel Corach	Systematic analysis of decadactylar subungueal genetic traces in murder cases
369	Andrea Gladys Colussi	Analysis of DNA from fingernail samples in criminal cases
370	Alice Squassina	Genetics 4N6FLOQSwabs are supporting DNA preservation from buccal swabs after long term storage
371	Dinah Aloraer	Evaluation of collection protocols for the recovery of biological samples from crime scenes
372	Pilar Martinez	Sexual aggressions sampling for semen with 4N6FLOQSwabs what is the outcome?
373	Bianca Szkuta	Potential degrading effect of sodium hypochlorite on exhibits containing DNA
374	Johannes Hedman	Crime scene DNA sampling by wet-vacuum applying M-Vac

Theme 16 Legal Medicine

375	Henrik Green	Genetic testing of sudden cardiac death genes in sudden unexpected deaths
376	Loredana Buscemi	Next generation sequencing of 68 genes in sudden unexplained death young individuals in forensics
377	Selena Cisana	Brugada syndrome genetic testing: a reliable Sanger sequencing protocol
378	Eleni Liapi	Effect of RNA integrity on reference gene expression stability in human post-mortem wounded and normal skin
379	Yu Kakimoto	Detection of miRNAs in tissue samples at autopsy: A trial for diagnostics at postmortem
380	Kristina Schwender	Analysis of DNA methylation patterns in sudden infant death syndrome
381	Beatrice Corradini	Estimation of the time of death through clock miRNA expression analysis in vitreous humor
382	Yadong Guo	Bacterial diversity in Bercaea cruentata gut described using Next Generation Sequencing
383	Jifeng Cai	Bacterial community succession analysis by Next Generation Sequencing in Changsha city, China
384	Jifeng Cai	The potential use of fungi community in postmortem interval estimation
385	Masaki Hashiyada	The comprehensive analysis of microRNA in hearts of stress model rat
386	Alina Glaub	Genetic variants in UGT genes

	Theme 17	Non-Human DNA
387	Jennifer M. Young	Residual soil DNA extraction improves biota characterization for forensic DNA analysis
388	Sherryn Ciavaglia	A novel forensic DNA profiling technique for protected species
389	Hiroaki Nakanishi	Identification of dog saliva using the mRNA expression assay
390	Weibo Liang	The species specific of 13 microRNAs in animal saliva
393	Rafal Skowronek	The presence of Diptera larvae in human bones
394	Anu Aaspollu	Advances in soil sample NGS data management for forensic investigation
395	Leigh Burgoyne	A study of one soil, its relatives and contaminant's by arbitrary primed PCR with 50-mer based analysis
396	Monique Wesselink	Anaysis of mtDNA control region from single cat hairs
397	Alexandra Summerell	Development of forensically informative DNA markers for Short-beaked Echidna - their utility in pedigree testing and application in wildlife trade
398	Burkhard Berger	The CaDNAP proficiency test - a quality control instrument for performance monitoring the forensic analysis of canine DNA
399	Gusztav Barany	Forensic identification of species by the sequence analysis of cytochrome b
400	Balazs Stégmar	Mitochondrial DNA control region variability in Hungarian canine breeds: a forensic application
401	Zsolt Padar	Increasing relevance of non-human genetics in Hungarian forensic practice
402	Anibal Gaviria	Development of a multiplex system for identifying individuals of Andean Condor (Vultur Gryphus)
403	Dayse Silva	Barcode analysis using mini-amplicons strategy for museum samples of the neotropical primate Callithrix spp
404	Hyehyun Oh	Development of the genetic markers using the whole chloroplast genomes for discriminating narcotic poppies
405	Andrea Verzeletti	Genetic approach for species identification: forensic application of DNA barcoding
406	Cesar Amaral	The shark panel: an InDel multiplex for shark species identification
407	Cesar Amaral	Identification of freshwater fishes from the South American Atlantic Rainforest: the case study of the Genus Phalloceros, using DNA barcoding and SPInDel approaches
408	Susana Jimenez	Big game species identification by High-Resolution Melting
409	Susana Jimenez	Efficacy of 12 STRs kit in cattle genotyping for forensic purposes
410	Zhen Hu	Species identification through pyrosequencing 12S rRNA gene
411	Yadong Guo	Application of mtSNP marker for genetic identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) in China

	Theme 18	Quality Control
412	Erica Romsos	Certification of a DNA quantitation standard using digital PCR
413	Koro Fernandez Oliva	Accreditation of the GHEP-ISFG Proficiency Test: One step forward to quality assurance and improvement of this Forensic Intercomparison Exercise
414	Tobias Poehlmann	DNA contaminations versus complex DNA traces
415	Ulrich Neuhaus-Steinmetz	eDNA - an expert system to speed up forensic DNA casework
416	Heather MacIntosh	The virtual DNA scientist: how two crime labs used automation to increase DNA scientist sample output by more than 40%
417	Susanne Lunoee Friis	Results of the 2015 relationship testing workshop of the English Speaking Working Group
418	Juan Jose Builes	Colombian results of the interlaboratory quality control exercise 2013-2014
419	Julianne Henry	A survey of environmental DNA in South Australian Police laboratories
420	Zsolt Padar	Accreditation of forensic laboratories as a part of the ,,European Forensic Science 2020" concept in V4 Countries
421	Miguel Marino	Experience in forensic genetics interlaboartory collaborative quality control exercises in Argentina (1998-2014)
422	Carolyn Steffen	Update of NIST SRM 2391c: PCR-based DNA Profiling Standard: What is new?
423	Elizeu Carvalho	Profile mapping DNA laboratories overlooking the forensic field and the use of quality systems

Theme 01: Population Genetics

1. GENETIC SIMILARITY SCALE OF TANDEM REPEAT VARIABILITY IN THAI HUMAN GENOME

Chaveerach A.[1,2], Sudmoon R.[1,2], Tanee T.[2,3], Yaipool K.[4], Sanubol A.[1], Thooptianrat T.[1], Yimpongsawat N.[1], Faijaidee W.[1]

[1] *Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand*
[2] *Genetics and Environmental Toxicology Research Group, Khon Kaen University, Khon Kaen, Thailand*
[3] *Faculty of Environment and Resource Studies, Mahasarakham University, Mahasarakham, Thailand*
[4] *Faculty of Law, Khon Kaen University, Khon Kaen, Thailand*

rsudmoon@yahoo.com

Short Tandem Repeats (STRs) also called microsatellite or Simple Sequence Repeat (SSRs) have been used for human identification and parentage testing in forensic science. For the studying on genetic variation level in Thais, the hair shafts with roots of 192 individuals were collected from southern, northern, central and northeastern Thailand. The analysis was done using seven STR loci namely D1S80, TPOX, D3S1358, D5S818, CSF1PO, TH01 and D21S11. The total bands counted from five percentage polyacrylamide gel electrophoresis were 650 bands with 326 characters. The total bands were used for dendrogram construction produced similarity indices (S) of a human species of Thais. The S ranges between 0.87 and 0.98 implied to genetic variations of 0.02-0.13 in a Thai human species. These values will be further advantaged for forensic evidence as race identification.

2. POTENTIAL STATISTICAL DIFFERENTIATION BETWEEN JAPANESE AND KOREAN POPULATIONS WITH ABOUT 100 STRS

Yamamoto T.[1], Hiroshige Y.[1], Ogawa H.[1], Yoshimoto T.[1], Mano S.[2]

[1] *Department of Legal Medicine and Bioethics, Nagoya University, Nagoya, Japan*
[2] *Department of Mathematical Analysis and Statistical Inference, The Institute of Statistical Mathematics, Tachikawa, Japan*

yamachan@med.nagoya-u.ac.jp

Introduction:

It is very interesting to estimate the biogeographical ancestor for each individual, and important to establish each identity genetically. In East Asians, it was reported decades of SNPs had made it possible to distinguish between Japanese and Chinese by PC analysis. For this procedure, however, comparatively much amount of DNA (> 500 ng) would be necessary. Alternatively, STRs are genetically neutral and more numbers of alleles. In this study, we tried to examine whether about 100 STRs with less amount of template DNA could differentiate between Japanese and Korean people genetically.

Materials and Methods:

1. Genotype data for 105 autosomal STR loci with each 32 individuals from 5 regional Japanese (Akita, Nagoya, Oita, Nagasaki and Okinawa) and 2 regional Korean (Seoul and Shenyang) populations were used for statistical analyses.
2. A category analysis was performed based on FCA (factorial correspondence analysis) using Genetix software.
3. A discriminant function analysis was performed by a Bayes factor to quantify the likelihood ratios for Japanese to Korean.

Results and Discussion:

In FCA, almost all of 160 Japanese and 64 Koreans distributed in each a category

except a Japanese and a Korean. Among Japanese, 4 regional Japanese from the main-land and Okinawa-Japanese were classified into each a category except one Nagasaki-Japanese. When the cross-validation by leave-one-out method was performed, it was shown as general tendency that it was easier to determine Japanese-origin but more difficult to determine Korean-origin.

3. FORENSIC AND POPULATION GENETIC ANALYSES OF 20 STRS LOCI IN MENDOZA POPULATION

Locarno L.[1,2], Furfuro S.[1], Marino M.[1,2]

[1] Laboratorio de Análisis de ADN, School of Medical Sciences, National University of Cuyo, Mendoza, Argentina
[2] Laboratorio de Genética Forense, Poder Judicial de Mendoza, Mendoza, Argentina

mmarino@fcm.uncu.edu.ar

This study established allele frequencies and some parameters of forensic interest from a sample of 4707 unrelated individuals from Mendoza province in Argentina using 17 STRs included in the Powerplex-16 System and AmpFISTR Identifiler Amplification Kit (D3S1358, TH01, D21S11, D18S51, Penta_E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta_D, vWA, D8S1179, TPOX, FGA, D2S1338 and D19S433).

On the other hand, the PowerPlex 21 System is a new kit developed for forensic laboratories, but there was a lack of data about this kit in Argentina. This kit contains, besides the above 17 loci, 3 new, D1S1656, D6S1043 and D12S391.

In order to evaluate this kit and to get basic population data for its use in forensic practice in Mendoza population, 397 unrelated individuals of the total were typed using this kit.

No deviations from Hardy–Weinberg equilibrium were observed using the Bonferroni correction for the number of loci analyzed.

The most informative loci in our data set was the Penta E, with discrimination power larger than 0,98 and typical paternity index of 5,43.

The combined matching probability value for 20 loci was 3,25E-26 vs 1,61E-21 for 17 STRs, this shows that these 3 extra STRs offer high effectiveness for forensic application.

Genetic distance between our sample and data from 15 other provinces previously published show the greatest distance between Mendoza and Patagonian (Rio Negro and Chubut) and Altiplano samples (Salta).

The creation of local reference databases it is extremely important, so this represents an important contribution to the local forensic genetics.

5. ANALYSIS OF THE GENETIC STRUCTURE OF SANTA CRUZ PROVINCE AND IT COMPARISON WITH THE OTHER SOUTHERN PATAGONIAN PROVINCES OF ARGENTINA

Cano H.[1], Ginart S.[2]; Caputo M.[2,3], Corach D.[2,3], Sala A.[2,3]

[1] Laboratorio Regional de Investigación Forense, Poder Judicial de Santa Cruz, Argentina
[2] Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry Universidad de Buenos Aires, Argentina
[3] Consejo Nacional de Investigaciones Científicas y Tecnológicas-CONICET, Argentina

dcorach@ffyb.uba.ar

Santa Cruz is the southernmost continental Patagonian province (latitude ranging 46°00´ S - 52°23´ S and longitude 65°43´ W - 73° 35´ W). Its demographic history is complex and changing over time. The oldest settlers were the Aónikenk, arriving around 12000 years before present. The Mapuche penetration started from western Andes during the seventeenth and eighteenth centuries. Europeans arrived during nineteenth and early twentieth centuries. Since then, the region experienced a constant demographic fluctuation due to Argentinean and international migrations.

Aiming to investigate the overall genetic features of forensic interest in the Province of Santa Cruz a total number of 645 individuals were selected. The sample set included: Santa Cruz (N=145), Chubut (N=102), Río Negro

(N=99), Buenos Aires (N=93) and potentially parental population samples from CEPH (N=206). Analysis included: 15 autosomal STRs, 23 Y-STRs and a set of 6 Y-SNPs. Genetic distance was significant between Santa Cruz either to Chubut, Rio Negro or Buenos Aires populations. Native American Y-specific haplogroup Q1a3a was present in around 15% of the Santa Cruz males and the haplogroup R1b was the most frequent European hg (45%). Structure analysis demonstrated that Santa Cruz population is composed by three ancestral contributors: European (50%), Native American (36%) and African (13%). Genetic characterization of this population allows increasing genetic knowledge of the country, in which previous studies have identified regional differences associated with the complex demographic process that took place in South America.

6. MALE ANCESTRY OF AFRO DERIVED POPULATIONS OF ALAGOAS, BRAZIL, DETERMINED BY ANALYSIS OF 24 Y CHROMOSOME SNPS

Azevedo D. A.[1], Santos J. C.[1], Araujo I. S. N.[1], Santos M. D.[1], Souza G. R. B.[1], Silva L. A. F.[1]

[1] Institute of Biological and Health Sciences, Federal University of Alagoas, Maceió, Brazil

laferreirasilva@uol.com.br

Quilombolas are small afro derived groups, which are remnants of resistance movement against slavery in Brazil. In the State of Alagoas there are 64 Quilombolas populations officially recognized.In the present study was investigated the paternal ancestry in five Quilombolaspopulations of Alagoas yet not studied. Sample was comprised by 296 men with the following distribution by population: Pau d'Arco(PD) – 69;Quilombo (QB) –51; Vila Santo Antônio (SA) –46;Tabuleiro dos Negros (TN) –60; and BomDespacho(BD) - 70. Haplogroups were defined through the analysis of 24 Y-chromosome SNPs. Genotyping was performed by minissequencing with SNaPshotkit.Statistical analysis was made with Arlequin software. In all populations most male lineages belong to European haplogroups. The African haplogroup E1b1a1-M2 was observed at the following proportions: QB, 0.2157; TN, 0.1500; PD, 0.2174; BD, 0.1429; and SA, 0.0435. The Amerindian Q1a3a-M3 lineage occurred only in PD (0.014) and QB (0.118).The AMOVA test showed that these populations are genetically heterogeneous (F_{ST}= 0.0886, $p < 0.00001$). Significant pairwise genetic distances (F_{ST}) were observed, exceptfor the pairs QB/TN, QB/PD, QB/BD and TN/BD. The predominance of European paternal lineages observed between the Quilombolascan be attributed to European domination model during the colonization of Brazil. However, in PD, QB, TN and BD, African lineages weremost frequents than in the admixed population of Alagoas. This results points to a most expressive participation of African men to formQuilombolas populationsthan for the admixed population of Alagoas.

7. POPULATION GENETIC DATA OF 21 STR MARKERS IN SAUDI POPULATION

Khubrani. Y.

General Administration of Forensic Evidence

y.m.y.k@hotmail.com

This study is considered as the first comprehensive population genetic study of the Saudi population for forensic applications, which cover main regions in the country. The Kingdom of Saudi Arabia is the second largest Arabic country in terms of land area; which constitutes 80% of Arabian Peninsula. The country is populated with around 30 million individuals among of them 20 million are Saudi according to the government estimation.

A total of 500 blood samples were collected from healthy adult individuals on FTA cards after signing an informed consent. The samples were collected equally from the five main geographical regions in the country including Central, Northern, Southern, Eastern, and Western region.

Allele frequencies for 21 autosomal STRs were observed using GlobalFiler® the new life technologies kit. These markers namely are D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391 and D2S1338). Statistical parameters of forensic interest were estimated. Furthermore, analysis of Fst distance was estimated between the near relative populations. This study confirms that these loci are considered to be a valuable tool in forensic DNA testing especially in paternity testing of the Saudi population for their high discrimination power.

8. ANALYSIS OF ADMIXTURE IN NATIVE AMERICAN POPULATIONS FROM COLOMBIA

Ossa H.[1,2], Aquino J.[3], Sierra S.[4], Ramírez A.[5], Carvalho E. F.[3], Gusmão L.[3,6,7]

[1] Pontificia Universidad Javeriana, Facultad de Ciencias, Bogotá, Colombia
[2] Laboratório de Genética e Biología Molecular, Bogotá, Colombia
[3] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Brazil
[4] Departamento de Biología, Universidad del Tolima, Tolima, Colombia
[5] Universidad de Pamplona, Pamplona, Norte de Santander, Colombia
[6] Institute of Pathology and Molecular Immunology from University of Porto, Portugal
[7] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

lgusmao@ipatimup.pt

The current Colombian population is the result of genetic admixture between Native Americans, Europeans and Africans. Around 82% of the population is considered admixed, 15% are Afro-Colombians and just 3% are Native Americans. There are about 81 native groups in Colombia. In this work, a sample of 121 non-related individuals from three Native American groups were analysed for 46 ancestry informative InDel markers. One of these groups was the Pijaos (n=28) that originally belong to the Karib linguistic group and, because of their combative nature, they were almost exterminated during European conquest and colonial times. The other two studied groups belong to communities that have been less subjected to admixture with non-Natives. The Barí (n= 56), known as "Motilones" or "Dobukubi", is a Native group that inhabits the Serranía del Perija, Norte de Santander, since pre-Colombian times. They still keep their original language, the Barí-ara, which belongs to the Chibcha family. We have also studied a sample of Natives from Guainía (n= 37), formed by different groups that migrated from the Amazonia and Orinoquian regions, including the Desana, Curripaco, Puinave, Cubeo, Guaunano and Tucano, all belonging to Tucano and Arawak linguistic groups. This study allow determining genotypic and allelic frequencies for 46 ancestry informative InDels and to estimate Native American, European and African admixture proportions in three Colombian native communities. The results showed a very low European and African admixture in the Barí and Guainia native groups in contrast to the high levels of admixture presented by the Pijao.

9. THE NEAR EASTERN COMPONENT IN PRESENT-DAY TUSCANS FROM THE 1000 GENOME PROJECT

Gómez-Carballa A.[1,2], Pardo-Seco J.[1,2], Amigo J.[1,2], Martinón-Torres F.[2,3], Salas A.[1,2]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain
[2] Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain
[3] Pediatric Emergency and Critical Care Division, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain

antonio.salas@usc.es

Genetic analyses have recently been conducted on present-day Tuscans (Central Italy) in order to investigate their presumable recent Near East ancestry and their connection with the origins of the Etruscan civilization. We retrieved mitogenomes and genome-wide SNP data from 110 Tuscans (from the The 1000 Genome Project). We carried out diverse phylogeographic and evolutionary analyses using a large worldwide database of entire mitogenomes

(>26,000) and partial control region sequences (>180,000), revealing the presence of typical Near East haplotypes in Tuscans as isolated members of various mtDNA phylogenetic branches. The Near East component in Tuscan mitogenomes can be estimated at about 8%; a proportion that is comparable to previous estimates but significantly lower than admixture estimates obtained from autosomal data (21%). Phylogeographic and evolutionary inter-population comparisons indicate that the main signal of Near Eastern Tuscan mitogenomes comes from Iran. These results add further support to previously reported findings suggesting the presence of a significant Near East component in Tuscans. This recent Near East maternal component in present-day Tuscans do not show local or regional variation. This pattern is compatible with a demographic scenario with no founder events or bottlenecks and a recent arrival of Near Easterners to this region in Italy.

10. THE GENOMIC LEGACY OF THE TRANSATLANTIC SLAVE TRADE IN THE YUNGAS VALLEY OF BOLIVIA

Heinz T.[1], Cárdenas J. M.[1], Álvarez-Iglesias V.[1], Pardo-Seco J.[1], Gómez-Carballa A.[1], Santos C.[1], Taboada-Echalar P.[1], Salas A.[1]

[1] Unidade de Xenética, Instituto de Ciencias Forenses and Departamento de Anatomía Patoloxía e Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, Galicia, Spain

antonio.salas@usc.es

During the Transatlantic Slave Trade some Africans were forced to move to Upper Peru (Bolivia), at first to Potosí and later to the tropical isolated Yungas valleys. A total of 105 individuals from the Yungas have been sequenced for the mitochondrial DNA (mtDNA) control region, and mitogenomes were obtained for a selected subset of these samples. We furthermore genotyped 46 autosomal AIM-indels in order to investigate patterns of continental ancestry. MtDNA haplogroup composition in the Yungas differs from the remaining Bolivian population with 81% Native American, 18% African, and 1% European ancestry. Most African mtDNA haplogroups concentrate in the 'Afro-Bolivian' community Tocaña where a high African ancestry is also manifested in the Y chromosome (44%) and in the autosomes (56%). In sharp contrast to previous studies on the TAST, the ancestry of about 1/3 of the 'Afro-Bolivian' mtDNA haplotypes can be traced to East and South East Africa, which could be at least partially explained by the Muslim trade connected to the TAST. In addition, the gender bias observed in the Yungas not only affects the partitioning of major continental ancestries in the region; the data is also compatible with a larger introgression of African females than males in the Yungas gene pole.

11. MAPPING THE GENOMIC MOSAIC OF TWO 'AFRO-BOLIVIANS' FROM YUNGAS (BOLIVIA)

Pardo-Seco J.[1,2], Heinz T.[1], Taboada-Echalar P.[1], Martinón-Torres F.[2,3], Salas A.[1,2]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain.
[2] Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain.
[3] Infectious Diseases and Vaccines Unit, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain.

antonio.salas@usc.es

Unraveling the ancestry of 'Afro-American' communities is hampered by the complex demographic processes that took place during the Transatlantic Slave Trade and the (post-)colonization periods. 'Afro-Bolivians' from the subtropical Yungas constitute isolated communities that live surrounded by a predominant Native American community. By genotyping >580,000 SNPs in two 'Afro-Bolivians', and comparing these genomic profiles with data compiled from 57 African groups and other reference populations (n = 1,795), we aimed to disentangle the complex admixture processes undergone by 'Afro-Bolivians'. The data indicate that these genomes constitute a complex mosaic of ancestries that is 83-84% of African origin; the remaining 16-17% being

European and Native American. West-Central Africa contributed more than half of the African ancestry to 'Afro-Bolivians', and this component is related to populations living along the Atlantic coast (i.e. Senegal, Ghana, Nigeria). Contrary to expectations, the remaining 1/3 of their African ancestry could have originated in Southeast or East Africa, with the Luhya being closely related. Overall, the data indicate that the genome of 'Afro-Bolivians' was shaped by a complex process of admixture occurring in America between individuals originating in geographically distant populations from Africa; their genomic mosaics received additional contributions of Europeans and local Native Americans (e.g. Aymaras).

12. GENETICS PROVIDES NEW CLUES ON THE ETHNO-HISTORY OF THE MAYA AND THE 'LADINOS' FROM GUATEMALA

Söchtig J.[1,2], Álvarez-Iglesias V.[1], Mosquera-Miguel A.[1], Gelabert-Besada M.[1], Gómez-Carballa A.[1], Salas A.[1]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, 15872 Galicia, Spain
[2] Eurofins Medigenomix Forensik GmbH, Ebersberg, 85560 Bavaria, Germany

antonio.salas@usc.es

Genetic analyses on Native indigenous people from Mesoamerica are still limited. In Guatemala, individuals of Maya descent comprise 40% of the population, composing a diverse ethno-linguistic group with a rich history. We aim to characterize the population patterns of the Maya and make inferences about their past demography. Uniparental and Ancestry Informative Markers (AIMS) were examined in distinct Mayan populations samples along with data publicly available. All samples were sequenced for the control region (HVS-I/II) and genotyped for 29 coding region mtDNA SNPs. Furthermore, 12 Native American lineages were selected for entire mtDNA genome sequencing. Estimation of the coalescent times of the most recent common ancestor (TMRCA) was also computed. In addition, a set of 26 biallelic Y-SNPs was genotyped in males, and 46 AIM-InDel markers were assessed in order to infer biogeographic ancestry by applying different statistical tools. The data provide new insight into the demography and the ethno-history of the Guatemalans and the important role of Mesoamerica as a passageway between North and South America revealing a fluid gene flow in Mesoamerica and a prevalent unidirectional movement towards South America, most probably occurring during the Pre-Classic and Classic Eras of the Mayan chronology.

13. ANCIENT DNA ANALYSIS REVEALS ORIGINS OF 17TH-CENTURY ENSLAVED AFRICANS

Schroeder H.[1,2], Ávila-Arcos M.[3], Malaspinas A. S.[1], Poznik G. D.[4], Sandoval Velasco M.[1], Carpenter M. L.[3], Moreno Mayar J. V.[1], Sikora M.[1], Johnson P. L. F.[5], Allentoft M. E.[1], Samaniego J. A.[1], Haviser J. B.[6], Dee M.[7], Stafford T. W., Jr.[8], Salas A.[9], Orlando L.[1], Willerslev E.[1], Bustamante C. D.[3], Gilbert M. T. P.[1]

[1] Centre for GeoGenetics, University of Copenhagen, Copenhagen, Denmark
[2] Faculty of Archaeology, Leiden University, Leiden, The Netherlands
[3] Department of Genetics, Stanford University, Stanford, USA
[4] Department of Statistics, Stanford University, Stanford, USA
[5] Department of Biology, Emory University, Atlanta, USA
[6] Sint Maarten Archaeological Center (SIMARC), Philipsburg, St. Maarten
[7] School of Archaeology, University of Oxford, United Kingdom
[8] Department of Physics and Astronomy, Aarhus University, Aarhus, Denmark
[9] Departamento de Anatoma Patolxica e Ciencias Forenses, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

hschroeder@snm.ku.dk

We used genome-wide data to trace the origins of three enslaved Africans whose centuries-old remains were found on the Caribbean island of Saint Martin.[1] Between 1500 and 1850, more than 12 million Africans were transported to the New World as slaves. Historical records suggest that the vast majority were shipped from West and West Central Africa but their precise origins remain

largely unknown. We used a recently-developed whole-genome capture technique and Illumina sequencing to obtain partial genome sequences from the poorly preserved remains of three enslaved Africans that were found on the Caribbean island of Saint Martin. We then use PCA and ADMIXTURE analysis to compare the slaves' DNA with modern reference data from West and West Central Africa and to identify their mostly likely origins. We find that the three individuals had very different genetic backgrounds, suggesting that they were taken from different communities in Africa. While one of them clusters closely with Bantu-speakers from northern Cameroon, the other two show closer affinities with non-Bantu-speakers living in present-day Nigeria and Ghana. These findings provide the first direct evidence for the ethnic origins of enslaved Africans and demonstrate that genomic data provide another type of record that can shed new light on long-standing historical questions.

14. NEW GENOMIC INSIGHTS INTO THE WESTERNMOST EDGE OF THE EUROPEAN ROMANI DIASPORA

Gómez-Carballa A.[1,2], Pardo-Seco J.[1,2], Fachal L.[1], Vega A.[1], Cebey M.[2], Martinón-Torres N.[2], Martinón-Torres F.[2,3], Salas A.[1,2]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain
[2] Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain
[3] Pediatric Emergency and Critical Care Division, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain

antonio.salas@usc.es

Genome-wide SNP data and information on entire mitochondrial DNA (mtDNA) were obtained from Spanish Romani. The autosomal data show an average proportion of Indo-European component of 20%. Identity-by-State analysis pointed to its origin located in Northwest of India. Inferences done to the level of the mitochondrial DNA (mtDNA) correlates well with the autosomal data. Thus, phylogeographic analysis of mitogenomes revealed that haplogroup M5a1b1a1, of Indian origin, is the most distinctive European Romani haplogroup and represents 18% of their mtDNA pool. In a large worldwide database involving more than we observed only sporadic findings of M5a1b1a1 in non-Romani European individuals. This singular haplogroup was originated 1.5 thousand years ago in Northwest of India. The phylogeny of Romani mitogenomes shows clear signatures of low effective population sizes and founder effects. Overall, these results suggest that cultural identity and relative isolation have allowed the Romani to preserve a distinctive genomic heritage, with some features linking them unequivocally to their ancestral Indian homeland.

15. ANCESTRAL GENETIC COMPOSITION IN A POPULATION OF SOUTH WESTERN COLOMBIAN USING AUTOSOMAL AIM-INDELS

Urbano L.[1,2], Portilla E. C.[1,2], Muñoz W.[3], Sierra-Torres C. H.[1,2], Bolaños H.[2,4], Arboleda Y.[1,2], Aguirre D. P.[5], Mendoza L.[5,6], Carmona V.[5,7], Afanador C. H.[5,8], Salgar M.[5,7], Gusmão L.[9,10], Builes J. J.[5,7]

[1] Human Genetic Laboratory, Universidad del Cauca, Popayán, Colombia
[2] InnovaGen Fundation, Popayán, Colombia
[3] Department of Surgical Sciences, Universidad del Cauca, Popayán, Colombia
[4] Department of Pathology, Universidad del Cauca, Popayán, Colombia
[5] GENES SAS Laboratory, Medellín, Colombia
[6] School of Health Sciences, Pontificia Bolivariana University, Medellín, Colombia
[7] Institute of Biology, University of Antioquia, Medellín, Colombia
[8] School of Medicine, University of Antioquia, Medellín, Colombia
[9] IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal
[10] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil.

jjbuilesg@une.net.co

The Colombian population, as most of the populations in Latin America, consists in the ancestral mix of Europeans, Native Americans and Africans, these mixing ratios can be determined by Ancestry-Informative Markers (AIMs), this markers allow us to observe stratification in genetic association studies. The aim of this study was to determine the mixing ratios in a population sample of the Department of Cauca, Colombia with three different origins (European, Native American and African) using a set of 46 AIMs insertion-deletion (AIM-INDELs). 500 individuals were recruited through informed consent, considering bioethical principles of the Helsinki declaration. Genotyping of 46 AIM-INDELs was performed using the QIAGEN Multiplex PCR kit; amplification products were visualized in FMBIO IIe Genetic Analyzer. Allele frequencies, the Hardy Weinberg and the evaluation of genetic distances (FST) were performed using the Arlequin v 3.5 software. Additionally, a multidimensional scaling was performed with the STATISTICA v7.0 software. Finally, the software STRUCTURE v 2.3.4 was used to determine the ancestral mixture. Genetic contributions of the three ancestral populations were stablished. The average composition of the population of the Department of Cauca was 48% for Native American, 39% for European and 14% for African. It showed that the ancestral composition of the Department of Cauca has a greater contribution of Native Americans and Europeans compared to Africans which could be explained by the European migrations during the conquest of America.

16. ANCESTRY BACKGROUND OF A POPULATION SAMPLE FROM BOLIVAR DEPARTMENT, COLOMBIA

Martinez B.[1], Builes J. J.[2,3], Aguirre D. P.[2], Mendoza L.[2,4], Afanador C. H.[2,5], Meza C.[1], Marrugo J.[1]

[1] Molecular Genetics Lab, Institute for Immunological Research, University of Cartagena, Cartagena, Colombia
[2] GENES SAS Laboratory, Medellin, Colombia
[3] Institute of Biology, University of Antioquia, Medellín, Colombia
[4] School of Health Sciences, Pontificia Bolivariana University, Medellín, Colombia
[5] School of Medicine, University of Antioquia, Medellín, Colombia.

beatri23@yahoo.com

The current Bolivar population is the result of admixture between the European settlers, African slaves and Native Americans, for this, individuals of this population may have different ancestry compositions. The sub region of the Montes de María, with important Native American influence and high mobility of its residents to urban areas in the north of the department, has contributed to changes in the admixtures observed in recent decades in Bolivar department. In order to know the ancestral composition in this region, we obtained the genetic profile of 46 ancestry informative Indels (AIM-InDels) of 78 individuals. The ancestral components were calculated according to the methodology described in [1]; using STRUCTURE v2.3.3 with a burnin length of 100,000 followed by 100,000 MCMC, with a K = 3, considering the ''Admixture Model'' and correlated allele frequencies. As parental populations were used the genetic profiles of 322 individuals (Africans: 105 Europeans: 158, Native Americans: 59) reported by Pereira et al. (PLoS ONE. 2012; 7: e29684. doi: 10.1371/journal.pone.0029684 PMID: 22272242). In addition, profiles of 42 Native American reported by Xavier et al (PLoS ONE. (2015); 10(3): e0120155. doi:10.1371/journal.pone.0120155), were used. All ancestry estimates were tri-hybrid; 21.5% of the contribution was African, 29.4% was Native American and 49.1% was European contribution. This finding was expected according with recently reports of internal migration pattern. Our findings will be useful to investigate the influence of ethnicity in genetic complex diseases, like asthma and other prevalent conditions. Support: University of Cartagena.

17. INDELS MARKERS SET AND ANCESTRY ESTIMATES IN A POPULATION SAMPLE FROM ATLANTIC DEPARTMENT OF COLOMBIA

Garavito G.[1], Martinez B.[2], Builes J. J.[3,4], Aguirre D. P.[3], Mendoza L.[3,5], Afanador C. H.[3,6], Egea E.[1], Marrugo J.[2]

[1] Research Group in Immunology and Molecular Biology, North University,

Barranquilla, Colombia
[2] Molecular Genetics Lab, Institute for Immunological Research, University of Cartagena, Cartagena, Colombia
[3] GENES SAS Laboratory, Medellin, Colombia
[4] Institute of Biology, University of Antioquia, Medellín, Colombia
[5] School of Health Sciences, Pontificia Bolivariana University, Medellín, Colombia
[6] School of Medicine, University of Antioquia, Medellín, Colombia

beatri23@yahoo.com

Ancestry Informative Markers InDels (AIM-InDels) show high allele frequency variation between ancestral populations and are useful to estimate individual and population ancestry. Our goal was to characterize the frequencies of 46 AIM-InDels in a population sample of 50 individuals belong to Barranquilla city, Atlantic Department in Colombia. The ancestral components were calculated according to the methodology described in [1]; using STRUCTURE v2.3.3 with a burnin length of 100,000 followed by 100,000 MCMC, with a K = 3, considering the ''Admixture Model'' and correlated allele frequencies. As parental populations, the genetic profiles of 322 individuals (Africans: 105 Europeans: 158, Native Americans: 59) [1] and 42 Native American individuals [2] were used. The contribution of each founder population was estimated; we found 24.7% of African, 27.6% of Native American and 47.7 % of European. These findings reveal different admixture histories of the studied population, and generate reliable ancestry estimates usefulness to investigate association between ancestry and genetic complex disorders. Support: North University.

1. Pereira R., Phillips C., Pinto N., Santos C., Santos S. E. Bd., Amorim A., et al. Straightforward inference of ancestry and admixture proportions through Ancestry-Informative Insertion Deletion multiplexing. PLoS ONE. 2012; 7: e29684. doi: 10.1371/journal.pone.0029684 PMID: 22272242
2. Xavier C., Builes J. J., Gomes V., Ospino J. M., Aquino J., Parson W., et al.: Admixture and Genetic Diversity Distribution Patterns of Non-Recombining Lineages of Native American Ancestry in Colombian Populations. PLoS ONE. (2015); 10(3): e0120155. doi:10.1371/journal.pone.0120155.

18. RESULTS OF 16 AUTOSOMAL STRS STUDIED IN POPULATION SAMPLES FROM TURKEY, IRAQ, LITHUANIA, SLOVENIA AND ALBANIA

Poulsen L.[1], Tomas C.[1], Drobnič K.[2], Ivanova V.[3], Mogensen H. S.[1], Kondili A.[4], Bunokiene D.[3], Jankauskiene J.[3], Pereira V.[1], Morling N.[1]

[1] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Frederik V's Vej 11, DK-2100, Copenhagen, Denmark
[2] National Forensic Laboratory, Police, Ministry of the Interior, Vodovodna 95, 1000 Ljubljana, Slovenia
[3] State Forensic Medicine Service under the Ministry of Justice of the Republic of Lithuania, Vilnius, Lithuania
[4] Subdivision of Biological and Biochemical Examinations and Analyses F.S.D. – Hellenic Police, Athens, Greece

lena.poulsen@sund.ku.dk

The AmpF*ℓ*STR® NGMSElect™ kit includes five 'new' loci specified in the recently expanded European Standard Set (ESS) together with the markers from the SGM Plus® kit and SE33. Population samples from Turkey, Iraq, Lithuania, Slovenia and Albania were typed for 16 autosomal STRs using the NGMSElect™ kit. The total number of samples typed was 656 and ranged from N=93 to N=200 for each population. The purpose of the study was to characterize the diversity of the 16 autosomal STRs in the investigated population samples and to assess their usefulness in forensic casework and population genetics. We provide allele frequencies, investigate the Hardy-Weinberg expectations, calculate typical maternity and paternity indices as well as match probabilities. The allele frequencies of the five population samples were compared to data available in the literature for geographically relevant populations studied for the same autosomal STR markers. Sample bias corrected F_{ST} pairwise genetic distances were obtained and are presented in MDS plots.

19. GENETIC POLYMORPHISM OF 22 AUTOSOMAL STR LOCI IN CHINESE HAN POPULATION

Li J., Luo H., Song F., Hou Y.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

profhou@yahoo.com, forensic@scu.edu.cn

Microreader™ 23sp ID system was a new STR multiplex system developed for forensic application, but there was a lack of population data in the Chinese Han population. This kit contained 21 non-CODIS STR loci (D6S477, D18S535, D19S253, D15S659, D11S2368, D20S470, D1S1656, D22-GATA198B05, D7S3048, D8S1132, D4S2366, D21S1270, D13S325, D9S925, D3S3045, D14S608, D10S1435, D12S391, D2S1338, D17S1290, D5S2500), one CODIS STR locus (D16S539) and the amelogenin locus. In this study, a population sample in Chengdu Han population was analyzed with the kit. Forensic genetic parameters and probability values of the Hardy–Weinberg Equilibrium (HWE) were calculated with software PowerStats v1.2 and Arlequin v3.5. Allele frequencies of the 22 STR loci and further forensic genetic parameters were obtained. The results suggested that Microreader™ 23sp ID system can provide informative polymorphic data for identification and parentage testing in Chinese Han population.

20. USEFULNESS OF POWERPLEX FUSION SYSTEM AS TYPING METHODOLOGY OF CODIS AND ESS GENETIC MARKERS IN A IMMIGRANT POPULATION FROM CABO VERDE IN LISBOA

Resende A.[1,2], Amorim A.[1,3,4], Vieira-Silva C.[1,4], Ribeiro T.[1,4], Porto M. J.[1,4], Costa Santos J.[1,2,4], Afonso Costa H.[1,4]

[1] Serviço de Genética e Biologia Forenses, Delegação do Sul, Instituto Nacional de Medicina Legal e Ciências Forenses, Lisboa, Portugal
[2] Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal
[3] Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
[4] CENCIFOR – Centro de Ciências Forenses, Coimbra, Portugal

mariajoao.porto@dcinml.mj.pt

Cabo Verde is located on the West African coast, discovered uninhabited by Portuguese explorers, colonized by European and African slaves and culturally characterized by a mixture of their settlers. Cabo Verde is one of the countries with highest number of emigrants living in Portugal.

In order to characterize this population, genetic markers included in CODIS and in ESS were amplified with PowerPlex® Fusion. To determine the usefulness of the kit, samples from 500 unrelated individuals from Cabo Verde, currently living in Lisboa, were studied. To implement the genotyping method at the Forensic Genetics Service (SGBF) we determined the minimum DNA concentration, the minimum number of PCR cycles and the minimum final volume of the PCR reaction to obtain a complete genetic profile. Stutters identification and its proportion, allelic imbalance within marker and reproducibility were determined.

Minimum concentration to obtain a complete genetic profile with PowerPlex® Fusion amplified in a PCR with 28 cycles in a final volume of 6.25µl is 0.04ŋg/mL. Stutters ratio varied between 0.02% (Penta E) and 10% (D2S1338). Allelic imbalance was not observed, variation within markers wasn't below 70%.

Results were compared with genetic profiles obtained by other kits used in routine (between common loci) verifying coincidence between them. There were no off-ladder alleles detected and it was possible to type microvariants which were not present at the allelic ladder of other PCR kits.

PowerPlex® Fusion allows simultaneous characterization of CODIS and ESS genetic markers, in an economically way, with greater sensitivity and solid results.

21. AUTOSOMAL INDELS DISTRIBUTION IN METROPOLITAN MANILA, PHILIPPINES

Magalhães M. C. A.[1,2,3], Amorim A.[1,2,3], Gusmão L.[1,2,4], Gorostiza A.[5,6], González-Martín A.[5], Pereira R.[1,2]

[1] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*
[2] *Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal*
[3] *Faculdade de Ciências, Universidade do Porto, Porto, Portugal*
[4] *Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil*
[5] *Departamento Zoología y Antropología Física, Facultad de Ciencias Biológicas, Universidad Complutense, Madrid, Spain*
[6] *Laboratorio Identificación Genética, GENOMICA SAU, Grupo Zeltia, Madrid, Spain*

aamorim@ipatimup.pt

The Philippines are a group of 7,107 islands situated in the Asia-Pacific region harboring a high diversity of cultures, languages and people due to various migration waves, different degrees of admixture between neighboring groups and genetic drift. In this work we studied the most populous region of the country – Metropolitan Manila – with a set of small autosomal insertion deletion (indel) polymorphisms so as to assess their genetic diversity and usefulness in forensic context. Using a single-tube multiplex reaction [1] we characterized 38 indels in 195 individuals born in the National Capital Region, allowing to establish an adequate allele frequency database. The overall genetic diversity revealed to be high (average heterozygosity= 0.40890), similar to previous studies in neighbor populations. No significant deviations from Hardy-Weinberg equilibrium were found ($p \geq 0.02998$) considering Bonferroni correction for multiple testing. Pairwise linkage disequilibrium tests failed to detect significant gametic association between markers (after Bonferroni correction) except for one pair (rs10688868–rs2308026; $p \leq 10^{-5}$) localized in different chromosomes, and therefore the effect on the use of the product rule is negligible. The combined power of discrimination reached 99.999999999997%, showing the usefulness of the indel set in identification studies, and the combined power of exclusion was 99.50%. Despite being a relatively low value when compared with standard STR kits, it is similar to other forensic binary marker sets and can prove useful as a complementary tool in kinship testing. In conclusion, this study shows that this simple indel multiplex can be useful for forensic applications in Metropolitan Manila, Philippines population.

1. Pereira R., Phillips C., Alves C., Amorim A., Carracedo A. and Gusmão L.*: A new multiplex for human identification using insertion/deletion polymorphisms*. Electrophoresis. 2009; 30: 3682-3690

22. F VALUES AND ADJUSTED LR IN BRAZILIAN POPULATIONS

Hessab T.[1,2], Schrago C. G.[2]

[1] *Instituto de Pesquisa e Perícias em Genética Forense, DGPTC/PCERJ, Rio de Janeiro, RJ, Brazil*
[2] *Departamento de Genética, UFRJ, Rio de Janeiro, RJ, Brazil*

thessab@pcivil.rj.gov.br

DNA typing is a powerful tool in criminal cases. After comparing genetic profiles, statistical methods are performed to calculate the likelihood ratios (LR) and assess the probability of DNA evidence under alternative hypotheses. This analysis is based on frequencies of previous population studies. Nevertheless, substructure and inbreeding affect the population allele frequencies. Therefore, it is important to consider these parameters when performing DNA analysis. In this way, this work aims to compute F_{ST} indices among Brazilian population as a whole and for each Brazilian state independently. Moreover, we compared the probability of occurrence of matching DNA profiles, considering Rio de Janeiro and Brazilian population frequencies and by adjusting the LR with the F_{IT} parameter, which makes possible to accommodate substructure and inbreeding simultaneously. For this calculation, we have used genetic profiles of 500 unrelated individuals from Rio de Janeiro population and allele frequencies previously reported. Our data suggests that some Brazilian states

exhibit significant F_{ST} values in comparison to the Brazilian population as a single database. Furthermore, when compared to non-adjusted Brazilian frequencies, the LR inferred from Rio de Janeiro genetic profiles and frequencies from the same population presented a better fit to the LR calculated with the Brazilian population frequencies adjusted with the F_{IT}.

23. POPULATION DATA FOR SE33 LOCUS IN UNITED ARAB EMIRATES ARAB POPULATION

Alghafri R.[1]

[1] *Department of Forensic Sciences and Criminology, Dubai Police G.H.Q., Dubai, United Arab Emirates*

r.alghafri@hotmail.co.uk

The high power of discrimination for SE33 locus makes it one of the most informative loci for forensic applications. Considering that locus finding its way to most of the new commercially available autosomal STR multiplexes assays, it is important to evaluate forensic parameters in UAE population before implementing kits including such locus. Here we present a genetic population study for SE33 locus in the United Arab Emirates (UAE) Arab population. 588 randomly collected samples were amplified using optimized in-house singleplex reaction. Forensic parameters including Matching Probability, Power of Discrimination, Paternity Power of Exclusion and Typical Paternity index were calculated using Arlequin v3.5 software. Results demonstrate SE33 competence in UAE population as same as in other populations. In comparison with previously published data of 15 autosomal STR loci for UAE population [1], SE33 locus was found to have the highest power of discrimination and therefore it demonstrates the potential usefulness for forensic applications in caseworks as well as in complex paternity cases.

1. Alshamali F., Alkhayat A., Budowle B., Watson N.: *STR population diversity in nine ethnic populations living in Dubai*. Forensic Science International, 2005; 152; Pages 267-279.

24. AN INVESTIGATION OF 21 INSERTION DELETION MARKERS IN UNITED ARAB EMIRATES POPULATIO

Alghafri R.[1], Almheiri M.[1], Ashehhi S.[1], Zaumsegel D.[2], Bohnert M.[2]

[1] *Department of Forensic Sciences and Criminology, Dubai Police G.H.Q., Dubai, United Arab Emirates*
[2] *Institute of Legal Medicine, Faculty of Medicine, University of Wuerzburg, Wuerzburg, Germany*

r.algahfri@hotmail.co.uk

Ancestry informative markers (AIMs) are considered a useful tool for forensic investigation as it might give a clue about an unknown stain donor with an absence of DNA profile match or suspects. Recently, a multiplex assay (21plex) has been developed to amplify 21 ancestry informative insertion/deletion markers (InDels) simultaneously [1]. In the current study we present a validation study of the 21plex assay and analysis of these markers in 100 samples from United Arab Emirates (UAE) Arab population. A comparison analysis with previously published data for the HGDP-CEPH sample set is presented. Results demonstrate the usefulness of using these markers in estimating ancestry proportions in this population as well as show the accuracy of classification compared to the global data set. Forensic parameters were determined for the UAE population using Arlequin v3.5 software.

1. D. Zaumsegel, M. A. Rothschild, P. M. Schneider: *A 21 marker insertion deletion polymorphism panel to study biogeographic ancestry*. Forensic Science International: Genetics. 2013; 7; 305-312.

25. ANALYSIS OF THE MOST EFFICIENT AUTOSOMAL STRS AND GENETIC DATA FOR THE LOCUS SE33 IN ECUADORIAN POPULATION

Gaviria A.[1], Vela M.[1], Morejon G.[1], Galarza J.[1], Castillo G.[1], Pastas V.[1], Burgos Figueroa G.[2], Paz-y-Miño C.[2], Zambrano A. K.[1]

[1] Laboratorio de Genética Molecular, Cruz Vital - Cruz Roja Ecuatoriana Quito – Ecuador
[2] Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito, Ecuador

anibalgaviria@hotmail.com

Twenty three autosomal STR markers were analyzed to define which are the most informative in forensic genetic cases in Ecuadorian population. In this study, we report the genetic data of 1400 and 1800 unrelated individuals for SE33 and other 22 STRs, respectively. The parameters analyzed were: polymorphism information content (PIC), heterozygosity, power of discrimination and power of exclusion. SE33 was the best marker for forensic identification purposes because it showed the highest: polymorphism information content (PIC) of 0.9334; heterozygosity of 0.9191; power of discrimination of 0.9969 and power of exclusion of 0,8345. Hence, we recommend its use in routine and complex paternity cases. After SE33, the subsequent most informative STR markers for Ecuadorian population were PENTA E, FGA, D1S1656 and D19S433, and the less informative markers were VWA, D22S1045, D2S441, TPOX and D3S1358.

1. Gaviria A., et al.: Twenty two autosomal microsatellite data from Ecuador (Powerplex Fusion). Forensic Sci. Int. Gene. Suppl. 2013; Volume 4: page e330 – page e333.
2. Jacewicz R., Jedrzejczyk M. and Berent J.: The most efficient STR loci in forensic genetics in population of central Poland. Forensic Sci. Int. Gene. Suppl. 2008; Volume 1:page 340 – page 342
3. Butler M. John, et al.: SE33 variant alleles: Sequences and implications. Forensic Sci. Int. Gene. Suppl. 2011; Volume 3: page e502 – page e503.
4. Butler M. John, et al.: The single most polymorphic STR Locus: SE33 perfomance in U.S. populations. Forensic Sci. Int. Gene. Suppl. 2009; Volume 2: page 23 – page 24.

26. ALLELIC FRECUENCIES AND FORENSIC PARAMETERS FOR MINISTRS D10S1248, D14S1434 AND D22S1045 (NC01) IN A SAMPLE FROM CENTRAL ANDEAN COLOMBIAN REGION

Burgos Figueroa G.[1,2], Restrepo Arango T.[2], Ibarra Rodríguez A.[2], Gaviria A.[3], Machado Rodríguez G.[2,4], Mora C.[5], del Pilar Lizarazo Quintero R.[5]

[1] Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito – Ecuador
[2] Grupo de Investigación Identificación Genética IdentiGEN, Universidad de Antioquia, Medellín – Colombia
[3] Laboratorio de Genética Molecular, Cruz Vital - Cruz Roja Ecuatoriana, Quito – Ecuador
[4] Grupo de Investigación de Biotecnología. Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín – Colombia
[5] Laboratorio de Genética, Instituto Nacional de Medicina Legal y Ciencias Forenses, Bogotá – Colombia

anibalgaviria@hotmail.com

Forensic DNA samples are commonly scarce, found in presence of inhibitors, degraded and/or mixed. When conventional STRs are used, some genetic profiles show PCR amplification problems, such as partial or absent genetic profiles. Aiming to help solving forensic cases at INMLCF (Instituto Nacional de Medicina Legal y Ciencias Forenses) in Colombia, NC01 triplex system (D10S1248, D14S1434 and D22S1045) was chosen for standardization and validation. Allelic ladders were constructed and validated by sequencing. Genotypes for 450 Central Andean Colombian from 6 regions were analyzed. Fst values were calculated and concluded that the analyzed regions behave as a metapopulation with no signs of subdivision (F_{st} = 0.001881 to 0.003901). Population and forensic parameters were estimated, no significant departures

from H-W equilibrium were detected and loci were in linkage equilibrium. No signs of homology were detected when NC01 was evaluated on 9 new world primate species. 25 forensic cases were typed with NC01 MiniSTRs Triplex, and useful results were provided in more than 50% of them.

1. Coble M. D. & Butler J. M.: Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA. J. Forensic Sci. 2005, 50: 43-53.
2. Butler J. M. & Coble M. D.: Authors' response. J. Forensic Sci. 2007, 52: 494.
3. Paredes M., Galindo A., Bernal M. et al: Analysis of the CODIS autosomal STR loci in four main Colombian regions. Forensic Sci. Int. 2003, 137: 67–73

27. MISSING POPULATION STUDIES ON NEW EUROPEAN STANDARD SET LOCI (ESS) AND ITS IMPACT ON POPULATION STATISTICS

Emmerova B.[1], Ehler E.[2], Vanek D.[1,3]

[1] Forensic DNA Service, Prague, Czech Republic
[2] Charles University in Prague, Faculty of Education, Department of Biology and Environmental Studies, Prague, Czech Republic
[3] Charles University in Prague, 2nd Faculty of Medicine, Prague, Czech Republic

barbora.emmerova@dna.com.cz

In April 2009, the European Network of Forensic Science Institute (ENFSI) voted to adopt five additional STR loci (D12S391, D1S1656, D2S441, D10S1248, and D22S1045) to the already existing European Standard Set (ESS) of seven STRs (TH01, vWA, FGA, D8S1179, D18S51, D21S11, and D3S1358). These 12 EU core loci are typically accompanied by D16S539, D2S1338, and D19S433 when amplified with commercially available STR kits (e.g. ESX/ESI17 or NGM SElect) [1]. The number of population studies comprising the new ESS loci does not cover all populations previously typed for „old" loci. The decrease of the number of population studies published may be caused by the changed policy of some journals that restrict the publication of this type of data. We summarized the population studies on the new ESS loci published up-to-now, sorted the data by populations and journals and discussed the impact on statistical calculations.

1. Butler J, Hill C (2012) Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. analysis 24.

28. EVALUATION OF DIPPLEX INVESTIGATOR IN EUROPEAN, ASIAN AND AFRICAN POPULATIONS

D'Amato M. E.[1], Hefke G.[1,2], Davison S.[1]

[1] University of the Western Cape, Forensic DNA Laboratory, Cape Town, South Africa
[2] South African Police Service, Biological Laboratories, Cape Town, South Africa

DIPplex Investigator (Qiagen) is the first commercial indel kit. Numerous population studies have been published for the European and Asian continent. Here we present a comparative study for DIPplex Investigator between population data collected in South Africa and published data for the European and Asian continents. Unsupervised cluster analysis using Structure (1) and MDS (Multidimensional Scaling) show the important intercontinental population structure detected with this kit. Population and forensic genetic parameters show higher PRM for European followed by Asians and African populations. The evaluation of the presence of null alleles with a ML estimator (2) indicated a high proportion (> 0.25) of null alleles for D97 in Africans. The evaluation of selection processes using the Fst outlier method (3) indicated positive selection acting upon locus D114. The inference of population of origin is explored with a cautionary note over the accuracy of the assignments and the possibility of using this information as forensic working hypothesis.

1. J.K. Pritchard, M. Stephens, P. Donnelly: Inference of population structure using multilocus genotype data. Genetics 2000, 155:945-959.
2. S. Kalinowski, M. L. Taper: Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics 2006,

7:991-995.

3. Beaumont M. A., Nichols R. A.: Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London B, 1996, 263: 1619-1626.

29. EVALUATION OF AMPFLSTR IDENTIFILER® LOCI WITHIN SOUTH AFRICAN AND BOTSWANA POPULATIONS

Ristow P. G., Tau T., Davison S., D'Amato M. E.

University of the Western Cape, Forensics DNA laboratory, Cape Town, South Africa

An investigation of the loci contained within AmpFLSTR® Identifiler® PCR Amplification Kit for use as a forensics tool for Southern African populations. In this study population data generated from South African populations (Admixed and Bantu population) as well as from Botswana (Bantu and Khoi-San) is compared to American (African, Caucasian and Hispanic) (1), Asian and European genetic data. The random match probability was found to be lowest in the Bantu populations, followed by Asian and highest in populations with European descent. The data was also subject unsupervised clustering analysis using Structure (2) and discriminatory analysis of component analysis (3) which expose a continental population structure within populations. Inference of continental origin of samples was explored as a secondary objective to aid with forensic investigations.

1. Butler J. M., Schoske, R., Vallone, P. M., Redman, J. W., & Kline, M. C. (2003). Allele frequencies for 15 autosomal STR loci on US Caucasian, African American, and Hispanic populations. *Journal of forensic sciences*, 48(4), 908-911.
2. Pritchard J. K., Stephens M., & Donnelly P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155(2), 945-959.
3. Jombart T. (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24: 1403-1405. doi:10.1093/bioinformatics/btn129

30. DEVELOPMENT AND VALIDATION OF AN ALLELIC FREQUENCY DATABASE FOR QATARI POPULATION USING 13 RAPIDLY MUTATING Y-STRS MULTIPLEX ASSAY

Almohammed E., Alghafri R.[1] , Goodwin W.[1], Hadi S.[1]

[1] *University of Central Lancashire, School of Forensics and Investigative Sciences, Preston, UK*
[2] *Ministry of Interior of Qatar, Doha, Qatar*

ekalmohammed@uclan.ac.uk

Differentiating male lineages using non-recombining Y-chromosomal genetic markers is highly informative for tracing human migration and for forensic studies. Recently, it has been shown that the level of male lineage resolution can be enhanced by analysing Rapidly Mutating (RM) Y-STRs. The aim of this study was to develop an allelic frequency database for Qatari populatiom to evaluate the resolution power of 13 RM Y-STRs. The 13 RM Y-STRs (DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a/b, DYS626, DYS627, DYF403S1a/b, DYF404S1, DYS449, DYS547 and DYS612) were amplified in a novel multiplex PCR assay. 209 samples from unrelated Qatari males were anlaysed. The overall haplotype diversity (HD) was 100%. It was found that the markers which contributed the most toward high HD were DYF399S1 and DYF403S1a/b. Together with their value for paternal male relative differentiation, these RM Y-STRs will be a valuable asset for forensic casework. AMOVA test was performed between Qatari population in comparison to Gulf countries, Middle East, and several worldwide population data sets. F_{ST} values were also calculated. Geography was found to account considerably for the pattern of population sub structuring. The RM Y-STR markers showed remarkable haplotype resolution power in the Qatari population, high gene diversity and sufficient robustness for a diverse range of applications.

Theme 02: X-Chromosome Variation

31. X-STRS AS A TOOL FOR MISSING PERSONS IDENTIFICATION USING ONLY SIBLINGS AS REFERENCE

Alzate L. N.[1], Agudelo N.[1], Builes J. J.[2,3]

[1] *Maestría en Criminalística y Ciencias Forenses, Universidad Libre, Santiago de Cali, Colombia*
[2] *GENES SAS Laboratory. Medellín, Colombia*
[3] *Institute of Biology, University of Antioquia, Medellín, Colombia*

jjbuilesg@une.net.co, genforense@genesltda.com

In cases of missing persons identification, where the reference samples are parents or offspring the use of autosomal STRs (A-STRs) is very useful, but when the reference sample is a sibling, usually, is necessary to supplement the analysis of the A-STRs with sex chromosomes markers (Y-STRs or X-STRs). Currently, forensic laboratories in Colombia have not registered studies to assess the utility of genetic markers on the X chromosome for such cases. In this study, 15 pairs of brothers from the Department of Antioquia were studied. DNA was amplified with PowerPlex® HS System (Promega) for A-STRs and the X-STR Decaplex Kit (Gusmão et al. 2008). The statistical evaluation of different sibling pairs was performed using the results of both markers. Of the 15 cases in this study; five reached the value of LR required using only A-STRs, four reached the value of LR only adding the X-STRs, and in the remaining 6 cases, LR was obtained with low values, even using the two types of markers simultaneously. The use of X-STRs to complement the A-STRs in cases of siblings as reference is useful due to the increase in the LR (likelihood ratio). Subsequently, the use of this combination of markers allows an increase in the probability of the identification of missing persons with siblings. Will be an interesting issue to expand the use of this kind of markers to cases with second degree relatives and increase the number of markers analyzed to achieved a higher LR value.

32. X-SNP TYPING OF DEGRADED DNA FROM FORMALIN-FIXED TISSUES BY MALDI-TOF MS

Yan L., Li L., Zhenmin Z.

Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science, Ministry of Justice, China, Shanghai 200063

Objective: To develop an available, high resolution X-SNP typing method for extremely degraded DNA from unbuffered Formalin-fixed human tissue. Method: Various tissues (heart, brain, liver, spleen, kidney, lung, stomach and intestine) from autopsy were immersed in unbuffered formalin under room temperature and sampling at regular intervals. DNA was extracted, quantified and qualified by QIAamp kit, Quantifiler kit and Identifiler kit respectively. 52 X-SNP loci showing independent inheritance and high polymorphisms in Chinese Han populations were selected and the primers were designed by MassARRAY Assay Design software. Multiplex PCR was carried out in four amplification reactions and the related polymorphic sites were analyzed by allele-specific primer extension and followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Results: All the amplicons of 52 X-SNP loci were shorter than 105bp. 98%-100% of X-SNP loci were detectable from tissues preserved in unbuffered-formalin for 37 days and 88.46%-100% for 44 days by the assay. While, less than fifty percent of intact STR loci could be detected with Identifiler kit when the tissues were preserved in unbuffered-formalin over 30 days. Conclusions: SNP typing method was a useful tool for extremely degraded DNA because of its high amplification efficacy to small DNA fragments less than 105bp.

33. DEVELOPMENT AND VALIDATION OF THE NEW INVESTIGATOR ARGUS X-12 QS KIT

Scherer M., König M., Bussmann M., Prochnow A., Peist R.

QIAGEN GmbH, Qiagenstrasse 1 40724 Hilden Germany

Mario.scherer@qiagen.com

Analysis of X-chromosomal STRs provides a powerful tool to complement autosomal and Y-chromosomal STRs in solving complex kinship deficiency cases. The Investigator Argus X-12 kit has been widely used for this purpose during the past years. In order to improve robustness and speed, and to facilitate analysis further, we recently developed an updated version of the assay.

The second version kit makes use of the recently developed Fast Reaction Mix 2.0, which allows short PCR cycling protocols and provides a high inhibitor tolerance. A standard 30 cycle PCR using 500 pg of template DNA now can be finished within about 70 min. A Quality Sensor has been added as an internal control to indicate if the PCR reaction has performed properly. D21S11 was included as an autosomal alignment marker. By comparing the genotype of the alignment maker between X-chromosomal and autosomal STR analysis done within a case, potential mix up of samples can be detected. No changes to the 12 X-chromosomal STR markers were made. Only minor changes to their primers were applied, e.g. to improve A-addition to the final PCR product and to obtain a cleaner fluorescent baseline. Additional SNP primers have been introduced to overcome null alleles due to primer binding site mutations of DXS10101, DXS10146 and DXS10148 that have been found at elevated frequencies within African populations (1). The allelic ladder has been complemented by 14 additional alleles.

We will show data of the development and validation of the Argus X-12 QS kit.

1. Elakkary S., Hoffmeister-Ullerich S., Schulze C., Seif E., Sheta A., Hering S., Edelmann J. and Augustin C.: Genetic polymorphisms of twelve X-STRs of the Investigator Argus X-12 kit and additional six X-STR centromere region loci in an Egyptian population sample. FSI Genetics. 2014; Volume: 11 page 26 – page 30

34. DEVELOPMENT OF A NEW 17 X-STRS MULTIPLEX FOR A HIGHER GENETIC RESOLUTION IN FORENSIC AND ANTHROPOLOGICAL STUDIES

Prieto E.[1], Baeta M.[1], Nuñez C.[1], Jimenez S.[2], de Pancorbo M. M.[1]

[1] BIOMICs Research Group, Lascaray Research Center, University of the Basque Country UPV/EHU. Avda. Miguel de Unamuno, 3. 01006 Vitoria-Gasteiz (Spain)
[2] Dpt. Pathology and Surgery (Legal and Forensic Medicine), University of Miguel Hernández, Elche, Spain

susana.jimenez@umh.es

The need for genetic systems with higher resolution power is an on-going process in forensic and anthropology studies. Currently, two of the most widely used X-chromosome STRs (X-STRs) multiplexes are composed by 10 and 12 markers. These numbers of markers included are a drawback for complex kinship testing cases where a higher resolution is needed. In addition, the large size of some amplicons difficult their application to degraded samples. Here, we present a new multiplex of 17 X-STRs with the aim of increasing the resolution power and applicability of the current markers systems. The newly proposed set includes 10 X-STRs of the GHEP-ISFG decaplex (DXS8378, DXS9898, DXS7132, DXS7133, DXS7423, DXS6809, DXS9902, DXS6789, GATA31E08, and GATA172D05) and 7 other X-STRs (DXS6801, DXS10075, DXS10079, DXS6799, DXS6807, DXS6800, and DXS6803). The design was optimized to provide high sensitivity and resolution for challenging samples (markers size <300bp, including 4 mini X-STRs). Validation experiments to evaluate its performance consisted of studies of sensitivity, stability and precision. Two control DNAs (9947A and 2800M) were analyzed in triplicate with quantities ranging 25pg to 30 ng, obtaining complete genetic profiles from 100 pg of DNA template. Multiplex stability was successfully tested obtaining full profiles in presence of humic acid and haematin inhibitors (until 250-300ng/µl and 150-300 µM respectively). In order to ensure the precision of the allele assignment an allelic ladder was developed. These preliminary results prove the sensibility and robustness of the new X-STR set, enabling a

high-resolution alternative to the current X-STRs multiplexes.

35. ALLELE AND HAPLOTYPE FREQUENCIES OF 12 X-STR LOCI OF INVESTIGATOR ARGUS X-12 KIT IN A POPULATION OF NORTHERN POLAND

Wysocka J.[1], Kapińska E.[1], Cybulska L.[1], Rębała K.[1], Szczerkowska Z.[1]

[1] *Department of Forensic Medicine, Medical University of Gdańsk, Poland*
ul. Dębowa 23, 80-204 Gdańsk, Poland

wysocka@amg.gda.pl

Allele frequencies of 12 STR loci of the Investigator Argus X-12 kit where obtained from 200 unrelated, healthy persons residing in the northern part of Poland (100 males and 100 females). The analysed loci: DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB and DXS10148 belong to four linkage group located on the X-chromosome. The haplotypes analysis for the loci was carried out. The Hardy-Weinberg equilibrum was performed on female and male samples for all twelve markers. Obtained values of biostatistical parameters of the set of investigated X-chromosomal markers proved their usefulness in forensic identification, paternity testing and genetic population studies.

36. BELARUSIAN EXPERIENCE OF THE USE OF FAMLINKX FOR SOLVING COMPLEX KINSHIP CASES INVOLVING X-STR MARKERS

Shyla A.[1], Borovko S. R.[2], Tillmar A.[3,4], Kuzub N. N.[2], Kotova S. A.[1], Tsybovsky I. S.[1], Rębała K.[5]

[1] *Scientific and Practical Centre of the State Committee of Forensic Examinations of the Republic of Belarus, Minsk, Belarus*
[2] *State Committee of Forensic Examinations of the Republic of Belarus, Minsk, Belarus*
[3] *Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden*
[4] *Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden*
[5] *Department of Forensic Medicine, Medical University of Gdansk, Gdansk, Poland*

s_borovko@mail.ru

Use of X-STR markers extends and gives additional possibilities to a forensic DNA expert for kinship analysis. FamLinkX is software for the calculation of likelihood ratios (LR) for kinship cases based on X-STR DNA profiles that takes linkage and linkage disequilibrium as well as mutations of X-STR markers into account. We calculated LR values for 8 different cases that fall into the three categories: 1) cases involving a mother, a daughter and a putative paternal grandmother (n=5); 2) a case involving two different mothers, two daughters and a putative paternal grandmother; 3) cases where two females were tested whether they are full-siblings or unrelated (n=2). Belarusian haplotype frequency database of 360 unrelated Belarusian males has been uploaded to FamLinkX. LR values for all kinship cases were calculated with different lambda values (λ=1, 10, 100), used for haplotype frequency estimation, to get the most conservative LRs. To demonstrate the ethnic sensitivity of X-STR markers and importance of the choice of the appropriate X-STR population database for LR calculation we calculated LR values for the same kinship cases using Belarusian and Swedish X-STR databases. LR values for the same kinship case could differ several to 6000 times depending on the chosen database. X-STRs genotyping lets us to determine relatedness successfully in all cases although the results of autosomal STR profiling and mitochondrial sequencing (for the cases where only the samples of putative full-siblings were available) were not sufficient to resolve family relationships issues. LR values were high enough in all cases.

37. RESULTS OF 12 X-CHROMOSOMAL STR MARKERS STUDIED IN POPULATIONS FROM TURKEY, IRAQ, LITHUANIA AND SLOVENIA

Poulsen L.[1], Tomas C.[1], Drobnič K.[2], Ivanova V.[3], Bunokiene D.[3], Jankauskiene J.[3], Pereira V.[1], Morling N.[1]

[1] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Frederik V's Vej 11, DK-2100, Copenhagen, Denmark
[2] National Forensic Laboratory, Police, Ministry of the Interior, Vodovodna 95, 1000 Ljubljana, Slovenia
[3] State Forensic Medicine Service under the Ministry of Justice of the Republic of Lithuania, Vilnius, Lithuania

lena.poulsen@sund.ku.dk

Analysis of the linage markers X-chromosomal STRs can play a crucial role in solving complex kinship cases involving at least one female individual. In order to perform calculations of probabilities in relationship testing, there is a demand for haplotypes frequency distributions in the relevant populations. The purpose of this study was to expand our forensic X-chromosomal database. Individuals from four different countries (Turkey, Iraq, Lithuania and Slovenia) were typed for 12 X-chromosomal STR markers using the Investigator® Argus X-12 kit. A total of 337 individuals were investigated, 67 from Turkey, 102 from Iraq, 110 from Lithuania and 157 from Slovenia. Here, we present the X-STR allele and haplotype frequencies for four defined linkage groups. Forensic statistical parameters for the X-chromosomal markers, e.g. typical paternity indices and matching probabilities, were calculated from both allele and haplotype frequencies for the four linkage groups. Allele and haplotype frequencies estimated for the investigated population samples were compared to published frequencies of relevant populations. Sample bias corrected F_{ST} distances were calculated from both allele and haplotype frequencies and presented by multidimensional scaling (MDS) plots.

38. PATERNITY TESTING OF TWO FEMALE SIBLINGS WITH INVESTIGATOR ARGUS X-12 KIT: A CASE WITH SEVERAL RARE MUTATION AND RECOMBINATION EVENTS

Hering S.[1], Haas S.[2], Grasern N.[2], Edelmann J.[3]

[1] Institute of Legal Medicine, University of Dresden, Germany
[2] Confidence DNA-Analysen GmbH, Vienna, Austria
[3] Institute of Legal Medicine, University of Leipzig, Germany

jeanett.edelmann@medizin.uni-leipzig.de

Stable haplotypes of closely associated X-STRs have proven to be a powerful tool in kinship analysis especially for cases when father/ daughter relationships are to be tested. We present a presumable easy case: A women wanted to know if their daughters have the same father or not. First results with Argus X-12 kit showed one exclusion in DXS10148 which could be explained by a paternal one-step mutation. But comparing haplotypes of the mother and the daughters there was one incompatibility in linkage group III. To explain this a maternal 3-step mutation in HPRTB or two mutation/recombination events in linkage group III must have occurred.

Later further family information was given: the two alleged fathers are related by their mothers. Therefore the exclusion chance decreases using only X-STRs. Analysis of 29 autosomal STRs gave high probability for being full siblings. Analysis of additionally 13 X-STRs showed no further exclusion of having the same father. Reconstruction of possible maternal haplotypes demonstrated that two recombination events in linkage group III are very unlikely. We presume that one maternal mutation in DXS10103 and one recombination event in linkage group III must have occurred together with one paternal mutation in DXS10148. This case shows that the use of X-STRs requires knowledge not only of allele and haplotype frequencies but also regarding mutation rates and rare recombination events of markers within linkage groups. In special cases it would be necessary to involve further X markers. Collaboration of the

laboratories will help to solve such problems.

39. GENETIC DATA OF 10 X-CHROMOSOME STR LOCI IN ECUADORIAN POPULATION

Zambrano A. K.[1], Morejon G.[1], Galarza J.[1], Pastas V.[1], Castillo G.[1], Vela M.[1], Gaviria A.[1]

[1] *Laboratorio de Genética Molecular, Cruz Roja Ecuatoriana – Cruz Vital, Quito, Ecuador*

anibalgaviria@hotmail.com

X-chromosomal STRs have proven to be an efficient complement in some complex paternity and forensic cases. In this work, we report results obtained of ten STR X chromosome markers (DXS8378, DXS9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7133, GATA172D05, GATA31E08, and DXS7423) from a sample of Ecuadorian men population. We typed 200 unrelated males from 19 provinces of Ecuador. Samples were processed following the recommendations of the GEP-ISFG collaborative study (2008). Our data showed that locus DXS6809 had the highest values of discrimination power (PD) and polymorphism information content (PIC) with 0,8051 and 0,7888, respectively. The population under study did not show deviation from linkage disequilibrium.

1. Gaviria A., et al: Genetic characterization of an X-STR decaplex on the population of Pichincha (Ecuador). Forensic Science International: Genetics Supplement; 2013, Volume 4: page e144 – page e145.
2. Gusmao Leonor, et al: A GEP-ISFG collaborative study on the optimization of an X-STR decaplex: data on 15 Iberian and Latin American populations. Int. J. Legal Med. 2008; The online version: (doi:10.1007/s00414-008-0309-4).
3. Gusmao Leonor, et al: Results of the GEP-ISFG collaborative study on an X-STR Decaplex. Forensic Science International: Genetics Supplement; Volume 1: page 677 – page 679.

40. GENETIC DATA OF 10 X-STRS IN AN AFRO-DESCENDANT POPULATION SAMPLE OF THE DEPARTMENT OF CHOCÓ - COLOMBIA

Lopez M.[1], Manrique A.[1,2], Aguirre D. P.[1], Mendoza L.[1,3], Bravo M. L. J.[1], Salgar M.[1,4], Puerto Y.[5], Gusmão L.[6,7], Builes J. J.[1,4]

[1] *GENES SAS Laboratory, Medellín, Colombia*
[2] *School of Medicine, University of Campinas, São Paulo, Brazil*
[3] *School of Health Sciences, Pontificia Bolivariana University, Medellín, Colombia*
[4] *Institute of Biology, University of Antioquia, Medellín, Colombia*
[5] *Directorate of Criminal Investigation and Interpol, Policía Nacional, Bogotá, Colombia*
[6] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal*
[7] *DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil*

jjbuilesg@une.net.co; genforense@genesltda.com

The markers used preferentially in forensic practice, especially in paternity testing and biological relationships, are the STRs located on the autosomal chromosomes (A-STRs). However, in the last two decades has increased the use of STRs linked to the X chromosome (X-STRs), specially when the A-STRs are insufficient, in particular cases of kinship analysis, when the offspring is female. In this research, a population genetic study was realized in a population sample of Afro-descendant of the Department of Chocó - Colombia using 10 X-STRs: DXS6809, DXS7423, GATA172D05, DXS6789, DXS9902, DXS7132, GATA31E08, DXS7133, DXS9898 and DXS8378. A total of 285 individuals not biologically related were analyzed. All individuals were born in Chocó - Colombia, and they gave informed consent for this study. All studied loci are in Hardy-Weinberg equilibrium and presented high levels of discrimination power (DP) in men and women, and a power of exclusion (PE) set higher than 99.99 % for both trios (father-mother-daughter) and duos (father-daughter). Finally, we performed a genetic distances analysis showing significant differences between this population and others like Latin American and Iberian

that were previously reported in the literature. The results of this study showed that the 10 X-STRs markers are suitable to be used in forensic genetics and that the Afro-descendant population of the Department of Chocó, Colombia is a genetically distinct population. Due this, is recommended the development of appropriate databases of genetic markers specifics for this population.

41. ANALYSIS OF 33 X- INDEL PANEL IN ARGENTINEAN POPULATION

Caputo M.[1,2], dos Santos S.[3], Corach D.[1,2]

[1] *Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina*
[2] *CONICET (National Scientific and Technical Research Council, Argentina)*
[3] *Laboratorio de Genética Humana e Médica, Departamento de Patologia, Universidade Federal do Pará, Belém, Pará, Brazil*

dcorach@ffyb.uba.ar

Polymorphic genetic markers located in the X chromosome might represent a robust complement for special forensic identification cases, especially when biological kinship deficiency cases are being investigates. We analyzed statistical forensic parameters of 33 X-chromosome InDels polymorphisms (X-InDels) in a sample of 188 individuals living in Argentina.

The panel composed of 33 X-InDels markers was amplified in a single multiplex PCR. Hardy-Weinberg Equilibrium was evaluated in females. Disequilibrium linkage was tested by an extension of Fisher Exact probability test on contingency tables, D' and Chi Square values for male haplotypes.

Regarding LD, six blocks containing two or three linked loci were detected in agreement with other publications. The criteria of minimum allele frequency >0.20 was not fulfilled in at least 13 out of 33 studied markers. The mean heterozygosity was 0.359, being in 15/25 higher than 0.3. The accumulated power of discrimination in females was 99,999999566%) being higher than in males 99,99957%. The mean exclusion chance in trios and duos was 98,975% and 99,992%, respectively. Some of these markers (24/33) were used to estimate interethnic admixture in Brazilian population detecting a positive correlation with parental population reference samples. This feature might explain the slightly low values of forensic parameters. Under this perspective, the analyzed marker set could be used more efficiently as ancestry detecting system than as an identification tool. Finally, if this panel is used for identification purposes, impact of LD for some loci representing linkage groups should be taken into account.

42. GENETIC DIVERSITY OF 12 X-CHROMOSOMAL SHORT TANDEM REPEATS IN JEWISH POPULATIONS

Ferragut J. F., Castro J. A., Ramon C., Picornell A.

Institut Universitari d'Investigacions en Ciències de la Salut (IUNICS) and Laboratori de Genètica, Departament de Biologia, Universitat de les Illes Balears. Palma de Mallorca, Spain

apicornell@uib.es

Jews can be traced back to populations occupying a small geographic area, in Middle East, several thousand years ago. Contemporary Jews comprise several communities classifiable according to the location where they developed. Among others, these include Mizrahim, who have always resided in Middle East; Ashkenazim, who lived in Central and Eastern Europe; Sephardim who, after their expulsion from the Iberian Peninsula, lived in other Mediterranean countries; and North African Jews. Chuetas are an isolated and inbred community, descending from Majorcan Sephardic Jews. Their peculiar history has kept the memory of their Jewish origin and has prevented their gradual assimilation into the general population [1; 2]. The present study analysed 343 X-chromosomes from five populations with Jewish ancestry, aiming to build an X-STR database, based on the markers included in the Investigator Argus X-12 kit (Qiagen, Hilden, Germany), for anthropological and forensic purposes. The highest variability was found in DXS10146 and DXS10135 (29 and 28 alleles, respectively, and observed heterozygosities between 0.8077 and 0.9744). New

alleles were described in DXS10134, DXS10019, DXS10148 and DXS10135 markers. Amongst the 152 males analysed, the 4 X-STR trios of linkage group 1–4 revealed 123, 95, 93 and 108 haplotypes, respectively. Overall values obtained for Power of Discrimination were high (>5.05E+08) in both females and males and Power of Exclusion ranged from 1.25E+05 (Mizrahim Jews) to 3.38E+05 (North African Jews). This work demonstrates that this set of X-STR is highly discriminating and, therefore, provides a powerful tool for solving complex kinship cases in Jewish populations.

1. Ben-Sasson HH: A history of the Jewish people. Cambridge:Harvard Universtity Press, 1976
2. Laub E and Laub J: El mito triunfante: Estudio antropológico-social de los Chuetas mallorquines. Palma de Mallorca, Spain: Editorial Miguel Font, 1987

43. X-CHROMOSOMAL HAPLOTYPE FREQUENCIES OF FOUR LINKAGE GROUPS IN A POPULATION OF ARGENTINA

Martinez G.[1,2], Schaller C.[1], Nazar P.[1], Brondani A.[1], Gomez del Rio N.[1], Bolea M.[2], Martinez Jarreta B.[2]

[1] Servicio de Genetica Forense, Poder Judicial de Entre Rios, Parana, Argentina
[2] Dpto. de Medicina Legal, Forense y Toxicología, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, España

mjarreta@unizar.es

DNA samples of ninety unrelated anonymized male individuals living in province of Entre Rios, Argentina, were genotyped using Investigator® Argus X-12 system (Qiagen) in the following loci localized in four linkage groups: DXS10148, DXS10135, DXS8378, DXS7132, DXS10079, DXS10074, DXS10103, HPRTB, DXS10101, DXS10146, DXS10134, DXS742 (3). Haplotype frequencies were calculated for each linkage group. The frequency of most common haplotype was 0.0333, 0.0556, 0.0444, and 0.0444 for haplogroups 1, 2, 3, and 4 (2), respectively. The combined power of discrimination was more than 0.99999999. The mean exclusion chance was 0.9999999 (trios). Investigator® Argus X-12 proved to be very useful for sibship determination, since its combined power of discrimination and mean exclusion chance values were relatively similar to LR for autosomal STR kit (1). This work presents the first haplotype frequency data for Investigator® Argus X-12 system in an Argentinean population.

1. Szibor R1, Krawczak M, Hering S, Edelmann J, Kuhlisch E, and Krause D.: Use of X-linked markers for forensic purposes. Int. J. Legal Med. 2003 Apr; 1 17(2): 67-74.
2. Nothnagel M, Szibor R, Vollrath O, Augustin C, Edelmann J, Geppert M, Alves C, Gusmão L, Vennemann M, Hou Y, Immel UD, Inturri S, Luo H, Lutz-Bonengel S, Robino C, Roewer L, Rolf B, Sanft J, Shin KJ, Sim JE, Wiegand P, Winkler C, Krawczak M, and Hering S: Collaborative genetic mapping of 12 forensic short tandem repeat (STR) loci on the human X chromosome. Forensic Sci. Int. Genet. 2012 Dec; 6(6): 778-84.3.
3. Edelmann J, Lutz-Bonengel S, Naue J, and Hering S: X-chromosomal haplotype frequencies of four linkage groups using the Investigator Argus X-12 Kit. Forensic Sci. Int. Genet. 2012 Jan; 6(1): e24-34.

44. ALLELE NOMENCLATURE IMPLICATIONS OF NEW HIDDEN SEQUENCE VARIANTS AT DXS10148, DXS10074 AND DXS10134 LOCI

Gomes I.[1], Brehm A.[2], Gusmão L.[3,4,5], Schneider P. M.[1]

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany
[2] Human Genetics Laboratory, University of Madeira, Funchal, Portugal
[3] DNA Diagnostic Laboratory (LDD), Institute of Biology, State University of Rio de Janeiro (UERJ), Brazil
[4] IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Porto, Portugal

[5] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

peter.schneider@uk-koeln.de

The amount of population and forensic genetic studies available for X-STRs supports the need for having a common and accurate nomenclature among laboratories allowing for better communication, data exchange, and data comparison. Simultaneous Sanger sequencing of rare/new or silent alleles of diverse groups from West Africa, Iraq and Guinea Bissau allowed the identification of, so far, previously undetected sequence variations in the frequently used loci DXS10148, DXS10079 and DXS10134 (included in the commercial Investigator Argus X-12 kit, Qiagen, Hilden, Germany).

At DXS10148 variation was detected at 4 bases downstream from the flanking region of the repeat motif (plus strand). The previous reported sequence $(AAGG\text{-}AAAG)_2$ has been detected for the first time as a varying $(AAGG\text{-}AAAG)_{1\text{-}3}$ motif in this present work. One additional string (totaling 3 copies of AAGG-AAAG) adds eight bases to the fragment size of the tetranucleotide STR. This means that 2 repeats are added in these cases when compared to the actual allele obtained by fragment size, while the presence of only one copy will reduce the expected allele size by 2 repeats. At DXS10074 two varying stretches consisting of CA and GA dinucleotide repeats were observed in the upstream flanking region, 5 bases from the main repeat core (plus strand) that have also influence on the expected allele size. DXS10034 revealed a new and simpler nomenclature in the Guinea Bissau sample set when compared to the previous described allele nomenclature. This detected new hidden variation also has impact in the actual allele repeat nomenclature at this locus.

Theme 03: Ethical and Legal Issues

45. FROM SUB-SOURCE TO SOURCE: INTERPRETING RESULTS OF BIOLOGICAL TRACE INVESTIGATIONS USING PROBABILISTIC MODELS

Maaskant-Van Wijk P. A.[1], Oosterman W. T.[2], de Zoete J.[1,2], Kokshoorn B.[1]

[1] Netherlands Forensic Institute, The Hague, The Netherlands
[2] Amsterdam University, Amsterdam, The Netherlands

p.maaskant@nfi.minvenj.nl

The interpretation of forensic investigations follows a hierarchy of propositions. From sub-source ('who is the donor of the DNA?') to source ('who is the donor of the semen in a sample?'), from source to activity ('how did the defendant's semen get on the victim's clothing?') and from activity to offense ('did the defendant rape the victim?').

The scientific community has already invested strongly in the interpretation and evaluation of results at subsource[1] and activity level[2]. Interpretation at source level has not yet received as much attention, although preliminary studies have addressed the association of body fluid testing and DNA results[3,4].

Interpretation of test results from biological trace investigations is complex. The presumptive tests that are commonly used display false positive and false negative rates and may show cross-reactivity in other human body fluids with the same proteins as tested for.

When attributing cell types to DNA donors, mixture ratios, gender specific cell types and masking of minor contributors need to be taken into account.

Here we present Bayesian networks that may assist in the interpretation and evaluation of test results and association of cell types and DNA donors.

Use of these types of models will promote correct evaluation, increase uniformity and reduce bias in reporting. Potential logical fallacies in

interpretation may be averted.

The practical use of these models is demonstrated using case examples.

1. Kelly H, Bright J-A, Buckleton JS and Curran JM: A comparison of statistical models for the analysis of complex forensic DNA profiles. Science and Justice. 2014; 54:66-70.
2. McKenna L: Understanding DNA results in the case context: importance of the alternative proposition. Frontiers in Genetics. 2013; 4, article 242:1-3.
3. Peel C and Gill P: Attribution of DNA profiles to body fluid stains. International congress series. 2004; 1261:53-55.
4. Harteveld J, Lindenbergh A and Sijen T: RNA cell typing and DNA profiling of mixed samples: can cell types and donors be associated? Science and Justice. 2013; 53:261-269.

46. WHAT DOES THIS STATEMENT REALLY MEAN?

Word C. J., Ph.D.

Consultant, Gaithersburg, MD USA

cjword@comcast.net

Since its introduction three decades ago, DNA testing has become the predominant forensic test where biological evidence is known to or assumed to have been deposited at a crime scene. Various parties in criminal investigations and trials have come to expect DNA test results to provide "the answer" or at least significantly aid in making appropriate determinations regarding a particular case. Sometimes the inclusion or exclusion of an individual as a DNA contributor at the crime scene is tantamount to determining the guilt or innocence of the individual. Historically, when full profile single source or two-person mixed DNA test results were reported, there was generally minimal confusion regarding the inclusion or exclusion of an individual regardless of the language and statistical probabilities used. Today, however, the bulk of DNA evidence results in partial low template DNA profiles often consisting of complex mixtures. The existing terminology in laboratory report wording guidelines is often not adequate to provide sufficient clarity regarding the results and conclusions obtained in a case. The introduction of new recommendations for interpretation and statistical calculations has exacerbated this problem. This presentation will focus on some of the confusing and often misleading language used for DNA test results, conclusions and opinions provided in laboratory reports and during testimony in court. Examples from actual cases from the United States where contradictory or misleading conclusions were reported will be presented along with recommendations for possible solutions and a discussion of the responsibility we have for accurate and informative interpretation and reporting.

47. BEYOND THE SOURCE: EVALUATING DNA EVIDENCE AT ACTIVITY LEVEL IN CASEWORK

Blankers B. J.[1], Aarts L. H. J.[1], Kokshoorn B.[1], Sjerps M.[1,2]

[1] Netherlands Forensic Institute, The Hague, The Netherlands
[2] Kortweg-de Vries Institute for mathematics, University of Amsterdam, the Netherlands

b.kokshoorn@nfi.minvenj.nl

The 'hierarchy of propositions' [Cook et al., 1998] distinguishes source level ('who or what is the source of the material?'), activity level ('what activity resulted in the findings?') and offence level ('what offence has been committed and by who?'). Evaluations at activity level require consideration of variables such as transfer and persistence of DNA as well as the prevalence of human cell material ('background DNA') in the specific circumstances of the case.

Traditionally in many countries forensic DNA experts report on source level and interpretation of the findings at higher levels is reserved for the judiciary. Assessing probabilities of transfer and persistence by non-experts may however lead to over- or under estimation of the weight of evidence. The added value of interpretations on activity level by DNA experts has therefore been acknowledged by both the forensic and judicial communities [Champod, 2013].

Until recently relatively few resources have been invested in tools and data for activity level interpretation and evidence evaluation by DNA experts. In part this is due to the complexity of these interpretations.

We will show how at the Netherlands Forensic Institute we currently address activity level questions from the courts in casework. We will also show how Bayesian Networks can be a useful interpretation tool for dealing with the large number of variables involved, as well as their conditional dependencies.

1. Cook R, Evett IW, Jackson G, Jones, PJ and Lambert JA: A hierarchy of propositions: deciding which level to address in casework. Science & Justice. 1998; 38: 231-239.
2. Champod C: DNA transfer: informed judgment or mere guesswork? Frontiers in Genetics. 2013; 4 (article 300): 1-3.

48. EXCLUSION RATES IN COURT-DIRECTED AND PERSONAL INFORMATION PATERNITY TESTS IN RUSSIAN FEDERATION

Zavarin V., Krassotkin E., Vinogradova S., Smirnova V., Kostinyuk G., Kalambet I., Kalambet N., Filippova A., Semikhodskii A.

OOO "Medical Genomics", Tver, Russian Federation

v.zavarin@medicalgenomics.ru

As part of a wider socio-economic study of paternity in the Russian Federation we have analysed exclusion rates in 3487 paternity tests performed in our laboratory in 2014. Out of this number 3220 tests were conducted for personal information and 267 tests were directed by courts. For all types of tests prior consent was obtained from participants. Both court-directed and personal information tests included duet (alleged father and child) and trio (alleged father, biological mother and child) cases.

The overall paternity exclusion rate was 23.5%. Exclusion rate for court-directed tests (19.1%) was somewhat smaller than that for personal information ones (23.9%) although no significant difference was observed between these values using χ^2-test ($p=0.05$). No significant difference between paternity exclusion rates in duet and trio personal information tests was also found (23.9% and 21.4% respectively). However, duet (28.6%) and trio (13.0%) cases for court-directed tests showed significant difference when analysed by χ^2-test ($\chi^2=10.044$, $p=0.002$). Our data indicate that paternity exclusion rates in Russian Federation are similar to those found for other countries (20-30%) when paternity was assessed by DNA analysis[1].

1. Annual Report Summary for testing in 2010. American Association of Blood Banks: 2011. Available at http://www.aabb.org/sa/facilities/Documents/rtannrpt10.pdf

49. MISINTERPRETATION OF SAMPLE CONTAMINATION IN A HUNGARIAN CASEWORK

Pádár Z., Kovács G.

Research Centre for Forensic Sciences and Criminology, University Széchenyi István, Győr, Hungary

The risk of contamination is a generally recognized and intensively researched field of forensic DNA (1-5). In spite of quality assurance systems, recommendations and regulations, this risk is always present even in routine casework as evidenced by this example from Hungary.

A couple - *J. K. M.* and *Z. K.* – were murdered and buried by *A. P.* and his accomplices between 03.27.2008. and 03.31.2008. During the investigation procedure suspicions arose that another missing person in the city – J. Gy. – might also be a homicide victim. On 07.20.2010. an unknown – *N. N.* – male body was discovered and exhumed.

In the process of individualization of the *N. N.* body, parts of the corpse and the living putative relatives of *N. N.* were analyzed with STR multiplex kits and CE separations in two different laboratories (A and B). The first analysis in "Lab A" used the soft tissues from N. N. bone sample. Based on the results, the first opinion excluded the biological relationship of N. N. with the putative relatives.

The subsequent analysis of "Lab B" used the soft tissue (muscle) parallel to the bone sample from the N. N. body. Based on the results, the second opinion supported the hypothesis of biological (maternal) relationship with high probability (W = 99,999%).

We examine and try to resolve the contradictions presented by the two interpretations, which originate from contamination of the remaining soft tissue.

1. Poy, A. L., van Oorschot, R. A. H. Trace DNA Presence, Origin, and Transfer within a Forensic Biology Laboratory and Its Potential Effect on Casework. Journal of Forensic Identification (2006) Vol.:56, Issue:4, 558-576.
2. Preuße-Prange, A., Renneberg, R., Schwark, T., Poetsch, M., Simeoni, E., von Wurmb-Schwark, N. The problem of DNA contamination in forensic case work – How to get rid of unwanted DNA? Forensic Sci Int Genet. (2009) Vol: 2, Issue 1, 185–186.
3. Ballantyne, K. N., Poy, A. L., van Oorschot, R. A. H. Environmental DNA monitoring: beware of the transition to more sensitive typing methodologies. Australian Journal of Forensic Sciences (2013) 45(3).
4. Szkuta, B., Harvey, M. L., Ballantyne, K. N., van Oorschot, R. A. H. The potential transfer of trace DNA via high risk vectors during exhibit examination. Forensic Sci Int Genet. Supplement Series (2013) 4(1):e55–e56
5. Szkuta, B, Harvey M. L., Ballantyne K. N., van Oorschot, R. A. H. DNA transfer by examination tools - a risk for forensic casework? Forensic Sci Int Genet. (2015) Feb 16; 16C:246-254. doi: 10.1016/j.fsigen.2015.02.004.

50. DNACTIVITY: INTERNATIONAL COOPERATION IN ACTIVITY LEVEL INTERPRETATION OF FORENSIC DNA EVIDENCE

Kokshoorn B.[1], Mc Kenna L. G.[2], Drotz W.[3], Ansell R.[3], van Oorschot R. A. H.[4], Aarts L. H. J.[1], Kloosterman A.[1]

[1] Netherlands Forensic Institute, The Hague, The Netherlands
[2] Forensic Science Ireland, Dublin, Ireland
[3] Swedish National Forensic Centre, Linköping, Sweden
[4] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Victoria, Australia A.D. A.D.

b.kokshoorn@nfi.minvenj.nl

Questions posed to expert witnesses by the legal community and the courts are expanding to include not just those relating to source level (i.e. 'who is the donor of the trace?') but also those relating to activity level (i.e. 'how did the DNA get there?').

To answer such questions within a probabilistic framework, empirical data is needed to estimate probabilities of transfer, persistence and recovery of DNA as well as background levels of DNA on everyday objects.

Laboratories that conduct such studies all use different experimental setups, trace recovery strategies and techniques and DNA analysis systems and equipment. It is essential for the forensic genetics community in general to establish whether the data generated by different labs are in concordance, and can therefore be readily used by the forensic community.

The aims and objectives of this ENFSI Monopoly 2013 project are to conduct a study of methodologies and data from different laboratories and to assess the comparability of the scientific data on transfer, persistence and recovery of DNA. This comparison will allow us to identify key factors that underlie potential variation. This information will be used to setup guidelines to enable sharing and database-storage of relevant scientific data. This will improve the ability of forensic scientists and other professionals of the Criminal Justice System to give evidence-based answers to questions that relate to the activity level of the crime under investigation.

This project is funded by the European Union TVEFS-2020 program.

Theme 04: Lineage Markers Abstracts

51. CONSTRUCTION AND APPLICATION OF MULTI-PLEX PCR FOR MTDNA SEQUENCING FROM HIGHLY DEGRADED AND SMALL AMOUNTS OF DNA

Minaguchi K., Miyashita K., Osawa M.

Department of Forensic Medicine, Tokai University School of Medicine

minaguci@tdc.ac.jp

Although mtDNA typing is useful for analyzing highly degraded DNA, we encounter more degraded samples from which even 200-bp sized PCR products of HV1 could not be amplified. To analyze these samples, we developed multiplex PCR of HV1 and HV2 regions with amplicon sizes of 120-155 bps and applied it to actual forensic cases. We divided HV1 and HV2 regions into 4 and 3 segments, respectively. Using the present two multiplex-PCR products as templates, we can directly sequence the 7 small segments. Multi-plex PCR can also decrease template DNA consumption. The sizes which could be determined from these PCR products were 78-115 bps. In order to cover most of the common lineages in East Asia, we set up the final sequence regions 16039-16401 for HV1, and 63-319 for HV2. To construct primers, we searched for the primer region not to affect amplification of the common haplogroups in East Asia. We further adjusted positions of the sequence regions of the 7 segments being readable only by forward primers, and being able to determine C-stretch samples by unilateral sequencing. The present system worked well in many samples for which Identifiler kit was completely unavailable. However, it additionally increased the detection sensitivity of contaminated DNA. In contaminated samples by more than 2 individuals, not all amplified fragments showed the concordant peak height ratios along with the amounts of contaminated DNAs. Therefore, the results must be judged in consideration not only with mtDNA haplogroups but also with such differences depending on sequencing reaction.

52. DEVELOPMENTAL VALIDATION OF THE YFILER PLUS PCR AMPLIFICATION KIT

Gopinath S., Zhong C., Nguyen V., Ge J., Carbonaro A., Mulero J.

180 Oyster Point Blvd 94080 South San Francisco, United States

Y-chromosomal markers have proven useful in solving investigations where low levels of male DNA are present in a high female DNA background. An intrinsic limitation of Y-STRs compared with autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome. Thus, in an effort to increase the power of discrimination we have developed a new 6-dye, 27-plex Y-STR system that includes the 17 markers from the AmpFℓSTR Yfiler® and Yfiler Direct kits plus 10 additional highly polymorphic Y-STR markers, including 7 rapidly mutating Y-STR loci which allow for improved discrimination of related individuals.

The new multiplex is a dual application assay designed to amplify DNA from extracted casework samples and database samples from storage cards and swab lysates via direct amplification. Compared to the previous Yfiler® and Yfiler® Direct kits, the new multiplex shows improved performance in inhibited samples and admixed male and female samples at ratios >1:1000, better differentiation in male:male mixture samples in high female DNA background, and faster time to results. The utility of rapidly mutating Y-STRs will be presented in the context of father-son studies and in studies performed with DNA extracted from low amounts of sperm cells.

Several thousand samples were collected with informed consent. The discriminating powers of Yfiler® and Yfiler® Plus are compared. The false positive and false negative rates of determining if two samples are from the same paternity lineage are investigated. Accordingly, the relevant interpretation guidelines for Yfiler® Plus matching will be developed.

53. HELENA'S MANY DAUGHTERS: MASSIVELY PARALLEL SEQUENCING PROVIDES FURTHER INSIGHTS INTO THE MOST COMMON WEST EURASIAN MTDNA CONTROL REGION HAPLOTYPE

Bodner M.[1], Strobl C.[1], Nagl S.[1], Xavier C.[1], Huber G.[1], Cardinali I.[2], Semino O.[3], Olivieri A.[3], Gandini F.[3], Achilli A.[2], Torroni A.[3],Parson W.[1,4]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[2] *Dipartimento di Chimica, Biologia e Biotecnologie, University of Perugia, Perugia, Italy*
[3] *Dipartimento di Biologia e Biotecnologie "L. Spallanzani", University of Pavia, Pavia, Italy*
[4] *Forensic Science Program, The Pennsylvania State University, PA, USA*

martin.bodner@i-med.ac.at

Mitochondrial (mt)DNA is a vital tool in forensic genetics when nuclear markers fail to give results or maternal relatedness is investigated. The ~1.1 kbp mtDNA control region (CR) displays highly condensed variation and is therefore routinely typed. In this restricted range, some samples share identical haplotypes and thus convey limited phylogenetic information and weak evidence in forensic queries. However, a matching CR does not imply that the linked coding regions are identical or that the mtDNAs belong to the same phylogenetic lineage. This is especially true for the most frequent West Eurasian mtDNA CR haplotype 263G 315.1C 16519C, which is observed in numerous clades within haplogroup H ("Helena") and occurs at a frequency of 3-4% in many European populations.

In a seminal study, we investigated the power of massively parallel complete (~16.6 kbp) mtGenome sequencing in 29 Italian samples displaying the most common West Eurasian CR haplotype. This allowed the detection of an unexpected high diversity with 28 distinct haplotypes clustering into 19 sub-clades of haplogroup H [1]. Here we present novel results from an ongoing investigation of a substantially expanded pan-Italian sample of almost 300 individuals with the "most common CR haplotype". This study demonstrates the benefit of complete mtGenome sequencing for forensic applications to enforce maximum discrimination, more comprehensive heteroplasmy detection, as well as highest phylogenetic resolution.

1. Bodner M et al.: Helena, the hidden beauty: Resolving the most common West Eurasian mtDNA control region haplotype by massively parallel sequencing an Italian population sample. FSI: Genetics. 2015; 15:21-26

55. INVESTIGATION INTO THE Y-STR TYPING USING NEXT GENERATION SEQUENCING

Kwon S. Y.[1,2], Kim E. H.[1,2], Lee H. Y.[1], Shin K. J.[1,2]

[1] *Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea*
[2] *Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Korea*

sykwon924@yuhs.ac

Next generation sequencing (NGS) can produce massively parallel sequencing data for many targeted regions at high depths of coverage, which implies the possibility of successful application of NGS to forensic casework sample analysis. In the present study, we evaluated the practical utility of NGS in Y-STR analysis using a new multiplex PCR system for 24 Y chromosomal markers including the PowerPlex Y23 loci and the M175 marker. The multiplex PCR system amplifies 24 fragments simultaneously in a size range of 85 to 250 bp using template-specific primers with read sequences, and the subsequent PCR reaction with primers having indices and platform-specific sequences produces barcoded library for the MiSeq® System (Illumina). With these simplified workflows, the 24 Y chromosomal markers were successfully typed using NGS in the samples mixed in a ratio of 1:1000 male and female DNA as well as 100 pg of single-source male DNA samples. In the analysis of more than 200 Koreans males, genotyping concordance between NGS and capillary electrophoresis method and sequence variations in targeted Y-STR region were scrutinized. Because the NGS with the developed multiplex PCR system uses small-size amplicons for commonly employed Y chromosomal markers and enables the detection of sequence variations, it will facilitate Y-STR analysis

from challenging casework samples such as degraded DNA samples and samples with multiple contributors.

56. MASSIVELY PARALLEL SEQUENCING OF MULTIPLEX SHORT AMPLICONS OF MTDNA FOR ANALYSIS OF CHALLENGED FORENSIC SAMPLES

Stoljarova M.[1,2], King J.[2], Churchill J.[2], Aaspõllu A.[1], Budowle B.[2]

[1] *Institute of Gene Technology, Tallinn University of Technology, Tallinn, Estonia*
[2] *Institute of Applied Genetics, University of North Texas Health Science Center, Fort Worth, USA*

monika.stoljarova@ttu.ee

Due to high copy number in human cells, mitochondrial DNA (mtDNA) has become a viable marker system for analyzing damaged, degraded and low quantity samples. Sequencing beyond hypervariable regions I and II (HVI and HVII, respectively) is rarely attempted, as the well-established methodology is laborious, time-consuming and costly. However, it has been reported that 75% of mtDNA variation resides outside of control region. The aim of this study is to develop a multiplex PCR assay comprised of short amplicons (≤ 200 bp-long) at targeted sites on the mitogenome using massively parallel sequencing (MPS). Nine regions within the coding region were chosen for multiplex primer assay design. *In silico* analysis showed that the addition of these nine regions can increase the discrimination power beyond sequencing of HVI and HVII by 21 % in Caucasians. For reference data complete mitogenomes of 114 Estonians were sequenced using the MiSeq platform (Illumina). The multiplex assay increased the discrimination power in the Estonian population by 9.1 %. Since only 87.7% of whole mtDNA haplotypes were unique, the Estonian mitogenome diversity can be considered relatively low. Previously established primers for HVI and HVII were added to the designed multiplex primer set for amplification of 170-210 bp long mtDNA fragments. The assay was further evaluated using Caucasian whole-blood and bone samples on the Personal Genome Machine (Ion Torrent, ThermoFisher). These results show that using MPS technology and additionally targeting 1.5 kb of mitogenome increases human identification discrimination power and improves the success rate of typing challenged samples.

57. RESULTS OF TYPING OF 71 BIALLELIC MARKERS ON Y CHROMOSOME IN XINJIANG HAN POPULATION OF CHINA

Li L.[1], Song Y.[1,2], Liu Y.[1], Lin Y.[1], Zhu R.[1], Zhao Z.[1]

[1] *Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science, Ministry of Justice, China, Shanghai 200063, China*
[2] *East China University of Political Science and Law, Shanghai 200042, China*

anneinchina@163.com

Objective: To screen out a panel of biallelic markers on Y chromosome that is informative in Chinese Han population in Xinjiang and valuable in forensic identification. Method: 71 Y chromosome-specific biallelic markers that are characteristic of major Asian haplogroups and subhaplogroups were selected. On Y chromosome, the loci were physically located at 2,655,180~24,359,931. Genomic DNA was extracted from saliva samples of 202 unrelated Chinese Han male individuals in Xinjiang. Typing of 71 biallelic markers was performed using the MassARRAY MALDI-TOF MS platform (Sequenom Inc.). Briefly, one 32-plex, one 31-plex and one 8-plex amplification reactions were processed following standard protocols for iPLEX chemistry. The reaction products were used as templates for the primer extension reactions and then spotted onto the MassARRAY SpectroCHIP. The target plate was inserted into the MALDI-TOF mass spectrometer of MassARRAY compact System and Y-biallelic markers loci was typed by MassArray TyperAnalyzer software version 4.0. Allelic frequencies, gene diversities (GD) for each locus were then determined. Haplotype diversity (HD) was also calculated. Results: Population data of 71 biallelic markers on Y chromosome were obtained. In Xinjiang Han population of China, out of the 71 tested Y-chromosomal markers, five loci (i.e. rs11575897, rs9341278, rs9306841, M148, rs2032645) were not polymorphic (GD=0),

13 loci were found to be low informative (0.01<GD<0.2), 20 loci were found to be medium informative (0.2<GD<0.3), 33 loci were found to be high informative (GD>0.3). Of 76 observed haplotypes, 61 (80%) were unique and 15 (20%) were found more than once. The total polymorphic markers giving high haplotype diversity (0.9930) demonstrate the utility of these loci for forensic identification. Conclusions: A panel of 53 ideal biallelic markers on Y chromosome was screened out. The markers may be used as supplement of autosomal STR loci and Y-STR loci for individual identification and relationship testing.

1. Jobling MA. Y-chromosomal SNP haplotype diversity in forensic analysis. Forensic Sci Int ,2001;118 (2/ 3) :158-162
2. Mengel-Jørgensen J, Sanchez JJ, Børsting C, et al. Multiplex Y chromosome SNP genotyping using MALDI-TOF mass spectrometry. International Congress Series. 2004; 1261: 15–17
3. Sobrino B ,Brin M ,Carracedo A. SNPs in forensic genetics: a review on SNP typing met hodologies. Forensic Sci Int , 2005; 154 (2/ 3) :181-194
4. Wei Wei, Hai-Bo Luo, Jing Yan, et al. Exploring of new Y-chromosome SNP loci using Pyrosequencing and the SNaPshot methods. Int J Legal Med, 2012; 126:825-833
5. Park MJ, Lee HY, Kim NY, Y-SNP miniplexes for East Asian Y-chromosomal haplogroup determination in degraded DNA. Forensic Sci Int: Genetics, 2013;7: 75–81
6. Mielnik-Sikorska M, Daca P, Marcin Woz´niak M, et al. Genetic data from Y chromosome STR and SNP loci in Ukrainian population. Forensic Sci Int: Genetics, 2013;7:200–203

58. ANCIENT DNA ANALYSIS TO UNCOVER MIGRATIONS IN MEDIEVAL TRONDHEIM

Kralj P.[1], Lundstrøm I. K. C.[2], Berger C.[1], Berger B.[1], Niederwieser D.[1], Nagl S.[1], Suppersberger Hamre S.[3], Parson W.[1,4]

[1] Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria
[2] Centre for GeoGenetics, Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark
[3] Department of Archaeology, History, Cultural studies and Religion, University of Bergen, Bergen, Norway
[4] Forensic Science Program, The Pennsylvania State University, PA, USA

Petra.Kralj@i-med.ac.at

The project "Immigration and mobility in mediaeval and post-mediaeval Norway" (http://medieval-immigration.b.uib.no/) is an international multidisciplinary research collaboration embedded in the Norwegian Research Council´s ten-year research programme "Cultural Conditions Underlying Social Change (SAMKUL)". The latter was established in response to the disastrous 2011 Norway attacks that caused uncertainty in the society questioning the own history and ethnical self-perception. A general lack of historical interest and a common belief that Norwegian society prior to 1970s was "ethnically" homogeneous have triggered the idea to combine historical information with archaeological, osteological, isotopic and genetic data obtained from medieval and post-medieval skeletal remains. These were excavated between 1972 and 2004 in Trondheim, the medieval capital of Norway. The remains are considered Norway´s best selection of well-documented human skeletal material representing different areas of the town as well as covering a wide time span (1175 to 1850). The molecular genetic part of the project aims at investigating the nature of pre-modern immigration by determining the geographic background of the individuals using linearly inherited markers. A total of 97 DNA-extracts were obtained from the archaeological remains (femora and teeth). Sex determination was performed using redundant gonosomal information (Amelogenin, SRY, X-STRs). Samples with male genotypes were further investigated for Y-STRs whereas the mtDNA control region was targeted in all samples. The composition of the linearly inherited haplotypes within the population examined is discussed in the light of the world-wide phylogenies to draw conclusions on migrations.

59. DEVELOPMENT OF A CONTROL REGION BASED MTDNA SNAPSHOT ASSAY

Weiler N.[1], de Vries G., Sijen T.[1]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*

Mitochondrial DNA (mtDNA) analysis is regularly applied in forensic DNA cases with samples having limited amounts of nuclear DNA (nDNA), such as hair shafts and bones. For mtDNA analysis of compromised samples, mini-mtDNA amplicons can be applied [1]. Despite the robustness, the method can be time- and cost consuming especially for cases with multiple samples. This has driven the development of a SNaPshot assay that uses mini-mtDNA PCR products as templates. Thus, the assay can be used as a screening tool to limit the number of samples taken through to Sanger sequencing on the remainder of the PCR product. The assay targets common mtDNA mutations in the control region.. More specifically, 18 single nucleotide polymorphisms (SNPs) are targeted, which were selected based on their relative frequency in a European population and a high discriminatory power in a Dutch population (97.2%). The assay was optimized and reliability testing showed no differences with Sanger sequencing results. Combining the mini-mtDNA method and SNaPshot assay is a forensic advantage and reduces the amount of DNA extract consumed, as the same PCR amplification product can be used for both screening of samples and confirmatory sequencing analysis.

1 C. Eichmann, W. Parson: 'Mitominis': multiplex PCR analysis of reduced size amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples, Int.J.Legal Med. 2008; 122: 385-388.

60. THE POLYMORPHISMS OF 23 DNA Y-STR LOCI IN TAIWAN RESIDENTS

Wu F.-C.[1], Yeh C.-J.[1], Chen M.-Y.[1], Chao C.-H.[1]

[1] *Department of Forensic Science for DNA Typing, Investigation Bureau, Ministry of Justice, No. 74, Zhong-Hua Road, Xindian District, New Taipei City 23149, Taiwan, R.O.C.*

wfcank@ms35.hinet.net

The buccal swab samples from 474 unrelated male volunteers were collected for typing Y chromosome DNA in Taiwan. Allele and haplotype frequencies for the 23 Y-specific short tandem repeat (Y-STR) loci of DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y GATA H4 (PowerPlex® Y23 System, Promega) were determined in the population. A total of 453 haplotypes were identified, of which 432 haplotypes were unique, and 21 haplotypes were found in two individuals. The haplotype diversity was 0.9977, and the discrimination capacity was 0.9557.

1. Wu FC and Pu CE: Multiplex DNA typing of short tandem repeat loci on Y chromosome of Chinese population in Taiwan. Forensic Sci Int. 2001; 120: 213 – 222.
2. Wu FC, Ho CW, Pu CE, Hu KY and Liu DH: Genetic polymorphisms of 17 Y-chromosomal short tandem repeat loci in Atayal population of Taiwan. Croat Med J. 2009; 50: 313 – 320.
3. Wu FC, Ho CW, Pu CE, Hu KY, Willuweit S, Roewer L and Liu DH: Y-chromosomal STRs haplotypes in the Taiwanese Paiwan population. Int J Legal Med. 2011; 125: 39 – 43.

61. IBERMITOBASE: A SEARCH TOOL FOR MTDNA POPULATION DATA IN THE IBERIAN PENINSULA

Asensio A., Marques S. L.[2], Ferragut J. F.[1], Alvarez L.[2,3], Picornell A.[1]

[1] *Institut Universitari d'Investigació en Ciències de la Salut (IUNICS) i Laboratori de Genètica, Departament de Biologia, Universitat de les Illes Balears, Palma de Mallorca, Illes Balears, Spain*

[2] *Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), Porto, Portugal*

[3] Faculty of Sciences of the University of Porto, Porto, Portugal

asentorr2012@gmail.com

Mitochondrial DNA (mtDNA) is a widely used genetic marker for population genetics studies and forensic purposes. In the population genetics field, mtDNA analysis plays a central role in investigating maternal lineages in the genetic landscape of extant populations and, together with the Y-chromosome, provides additional information concerning possible sex-biased genetic structure due to different demographic histories for males and females. In forensic casework, mtDNA is the tool of choice when DNA is present in a low copy number, is degraded, or to evaluate maternal relationships between individuals. Statistical interpretation of the results depends on mtDNA population data available and, undoubtedly, it is essential to consider geographical differences among populations for choosing suitable databases to weigh correctly the value of the evidence of an mtDNA profile match. Although many mtDNA studies focusing on autochthonous Iberian populations can be found in the literature (over 60 papers), the sequences of these data sets are not always available to the general scientific community and are therefore of limited use. To make these data accessible, here we present an online database with a compilation of the available mtDNA sequences of Spanish and Portuguese populations. Ibermitobase is an online tool (http://www.mtdnadatabase.hol.es) where users can easily search and download mtDNA sequences according to the following criteria: geographical region, population, mtDNA segment, haplogroup, and haplotype.

This database provides a valuable source of information for genetic structuring studies in Iberian populations and can be useful for a critical revision of the quality of published sequences for forensic practice [1].

1. Parson W and Bandelt HJ: Extended guidelines for mtDNA typing of population data in forensic science. Forensic Sci. Int. Genet. 2007, 1:13–19

62. 23 Y-STR DATABASE OF SAMPLES FROM SEXUAL ASSAULTS IN BRAZIL

Ferreira S. T. G.[1, 2], Paula K. A.[1], Maia F. A.[1], Svidzinski A. E.[1], Amaral M. R.[1], Diniz S. A.[1], Siqueira M. E.[1], Moraes A. V.[1], Purps J.[3], Gusmão L.[4, 5, 6], Roewer L.[3]

[1] Forensic DNA Research Institute, Federal District Civil Police, Brasília, Brazil
[2] National Secretariat of Public Security, Ministry of Justice, Brasília, Brazil
[3] Dept. Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité-Universitätsmedizin, Berlin, Germany
[4] Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil
[5] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal
[6] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

samueldoc83@yahoo.com.br

In sexual assault caseworks, the use of DNA databases may prove decisive in identifying perpetrators. Particularly, the analysis of Y chromosome STRs may play a crucial role in the study of the male portion in male/female cell mixtures, mixed DNA profiles from more than one perpetrator or from perpetrators and the sexual partners and azoospermic or oligospermic perpetrators. The analysis of Y chromosome specific markers can be also important in the study of the geographic ancestry of male lineages. The aim of this study was to genotype samples from sexual assaults in Brasília, Brazil, using the PowerPlex Y23 System and to construct a Y-STR database of these profiles in the Forensic DNA Research Institute of the Federal District Civil Police in Brasília in order to aid forensic casework. The samples are evidence collected from vaginal and anal swabs and clothing and from unrelated male perpetrators. A total of 600 were already typed for STRs and we found 75 serial rapists who attacked 210 women in Brasilia using a DNA database of STRs profiles of biological evidence from sexual assaults and rapes. In this work, we will present the results obtained concerning haplotype diversity and comparisons with other Brazilian population groups. The use of PPY23 provides high discriminatory

power, increasing the usefulness of Y-STR analysis in sexual assaults caseworks. In this study, the use of a 23 Y-STR database will substantially aid forensic investigations and will contribute to identifying more perpetrators.

63. RAPID AMPLIFICATION OF 13 RAPIDLY MUTATING Y-STRS IN A MULTIPLEX PCR ASSAY

Abuidrees A.[1,2], Alghafri R.[3], Hadi S.[2]

[1] Directorate of Forensic Science Evidence, Public Prosecution, Manama, Bahrain
[2] School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, United Kingdom
[3] Department of Forensic Sciences and Criminology, Dubai Police General Head Quarters, Dubai, United Arab Emirates

Aqeela.gx@gmail.com

Polymerase Chain Reaction (PCR) became the cornerstone of many developments in the molecular biology, especially within forensic DNA profiling. One of the early improvements was the application of multiplex PCR for amplifying Short tandem repeat (STR) markers. The efficiency of any multiplex PCR assay depends on optimal reaction components and PCR cycling parameters.

A multiplex PCR assay consisting of 13 Rapidly Mutating Y STR loci called RM-Yplex was developed at the University of Central Lancashire (UCLan). This assay has an amplification time of approximately 2.5 hours. This study aimed to investigate alternative DNA enzymes to decrease the PCR cycling time to the minimum with reliable results.

Phusion® Flash High Fidelity, TAKARA Z-taq™ and Platinum® Taq DNA polymerases were investigated for conducting RM-Yplex assay at various PCR cycling conditions. This study showed rapid, robust and efficient amplification for all of the RM YSTR markers within the multiplex with most enzymes when appropriate cycling conditions were used. The study also achieved in shortening the amplification time from 2.5 hours to less than 30 minutes with Phusion® Flash High Fidelity DNA polymerase using Verti® PCR machine. Comparisons of the multiplex PCR amplifications and their efficiencies using different enzymes are presented.

64. VALIDATION OF POWERPLEX Y23 SYSTEM (PROMEGA) USING REDUCED REACTION VOLUME

Barbaro A., Cormaci P.

Dept. Forensic Genetics
Studio Indagini Mediche E Forensi (SIMEF) - ITALY

simef_dna@tiscali.it

Y-chromosome specific STRs are commonly analyzed by forensic laboratories for paternity, genealogical testing and forensic caseworks, in particular in sexual assault cases where the identification of male specific DNA is essential in presence of mixed samples containing high female DNA quantity.

PowerPlex® Y23 System[1] is a 5-dye multiplex kit that analyze the 17 Y-STR loci commonly available in other kits (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4) together with 6 new discriminating Y-STR loci (DYS481, DYS533, DYS549, DYS570, DYS576, and DYS643) that have higher gene diversities.

Promega amplification protocol recommends a PCR total reaction volume of 25µL containing 5.0 µL Master Mix, 2.5µL Primer Mix, 7.5µL Grade Water and 10µL template DNA.

The purpose of the present study is to validate the PowerPlex® Y23 System using half volume reaction mix for the amplification of a wide range of forensic samples.

Results demonstrate the PowerPlex® Y23 System is a robust and sensitive kit capable of overcoming common inhibitors, giving reliable amplification results also with male/female DNA mixtures and low amounts of DNA template.

1. Thompson JM et al. Developmental validation of the PowerPlex® Y23 System: a single multiplex Y-STR analysis system for casework and

database samples. Forensic Sci Int Genet. 2013 Feb;7(2):240-50.

65. DEVELOPMENT OF AN OCTUPLEX CHRY MINISTR SYSTEM

He W.[1], Wang H.[2], Bai X.[2], Feng T.[2], Wang L.[1], Li Z.[1], Zhu W.[1], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China

liangweibo@scu.edu.cn

DNA samples from the crime scene were often highly degraded in forensic cases which would lead to partial results without the large sized STR loci. The reduction of STR known as miniSTR could facilitate the examination and analysis of degraded DNA samples. Lots of autosomal miniSTR panels have been developed while ChrY miniSTR system remains rare. Here, we developed a new ChrY miniSTR octuplex PCR set of 8 loci (DYS459, DYS16, DYS392, DYS437, Y-GATA-H4, DYS643, DYS439, and DYS438). These loci are present in Promega PowerPlex Y23 Kit with length between 263bp and 403bp. Primers of the 8 loci were redesigned for amplicons between 81bp and 243bp and labeled with 4 different fluorescence. Allelic ladder of the octuplex was amplified from the dilution of Y23 allelic Ladder with the octuplex primers. Artificial degraded DNA samples made by ultrasonic impact treatment, aged samples obtained from our lab and reference DNA samples were profiled with the octuplex and Y23 kit. Comparison studies showed that the octuplex can provide fully concordant results of reference samples to commercial STR kits and improved signal from artificial degraded and aged DNA samples. This new ChrY miniSTR panel will make it possible for forensic workers to use loci already present in commercial kits and genotype data that can be directly comparable to reference samples and searchable through now available databases in the examination and analysis of degraded DNA.

66. AN EVALUATION OF RAPIDLY MUTATING Y-STR MULTI-ALLELIC MARKERS

Alghafri R.[1]

[1] Department of Forensic Sciences and Criminology, Dubai Police G.H.Q., Dubai, United Arab Emirates

r.alghafri@hotmail.co.uk

Multi-allelic Y chromosome short tandem repeats (Y-STRs) have been avoided in forensic applications due to high level of complexity involved in the interpretation of these markers. Because of such reason Y-STR multi-allelic markers are have less representation in literature compared to Y-STR single-allelic markers. However these markers were proven to be highly polymorphic previously such as DYS464 [1]. In the recent discovery of the 13 rapidly mutating Y-STRs there were four multi-allelic markers including DYF387S1, DYF399S1, DYF403S1 and DYF404S1. In all subsequent studies these markers were always showing the highest diversity across the 13 RM Y-STRs [2, 3, and 4]. In this study these markers were investigated individually in 361 male individual samples from United Arab Emirates. Each marker was analyzed using previously published primers sets [5]. The potential forensic application value of these markers was evaluated using both conservative (C-type) and Extended (E-type) approaches.

1. John M. Butler, Richard Schoske, Forensic value of the multicopy Y-STR marker DYS464, International Congress Series, Volume 1261, April 2004, Pages 278-280.
2. Ballantyne, K.N., Keerl, V., Wollstein, A., Choi, Y., Zuniga, S.B., Ralf, A., Vermeulen, M., de Knijff, P., & Kayser, M. 2012. A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic Science International: Genetics, 6, (2) 208-218.
3. Robino, C., Ralf, A., Pasino, S., De Marchi, M. R., Ballantyne, K. N., Barbaro, A., Bini, C., Carnevali, E., Casarino, L., Di Gaetano, C., Fabbri, M., Ferri, G.,

Giardina, E., Gonzalez, A., Matullo, G., Nutini, A. L., Onofri, V., Piccinini, A., Piglionica, M., Ponzano, E., Previderè, C., Resta, N., Scarnicci, F., Seidita, G., Sorçaburu-Cigliero, S., Turrina, S., Verzeletti, A. &Kayser, M. Development of an Italian RM Y-STR haplotype database: Results of the 2013 GEFI collaborative exercise. *Forensic Science International: Genetics*.

4. Westen, A. A., Kraaijenbrink, T., Clarisse, L., Grol, L. J. W., Willemse, P., Zuniga, S. B., Robles de Medina, E. A., Schouten, R., van der Gaag, K. J., Weiler, N. E. C., Kal, A. J., Kayser, M., Sijen, T. & de Knijff, P. 2015. Analysis of 36 Y-STR marker units including a concordance study among 2085 Dutch males. Forensic Science International: Genetics, 14, 174-181.
5. Ballantyne, K.N., Goedbloed, M., Fang, R., Schaap, O., Lao, O., Wollstein, A., Choi, Y., van Duijn, K., Vermeulen, M., Brauer, S., Decorte, R., Poetsch, M., von Wurmb-Schwark, N., de Knijff, P., Labuda, D., Vuzina, H., Knoblauch, H., Lessig, R., Roewer, L., Ploski, R., Dobosz, T., Henke, L., Henke, J., Furtado, M.R., &Kayser, M. 2010. Mutability of Y-Chromosomal Microsatellites: Rates, Characteristics, Molecular Bases, and Forensic Implications. The American Journal of Human Genetics, 87, (3) 341-353.

67. COMPARISON BETWEEN YFILER AND RM Y-STRS IN UNITED ARAB EMIRATES POPULATION

Alghafri R.[1], Alhammadi S.[2], Alamiri K.[2]

[1] *Department of Forensic Sciences and Criminology, Dubai Police G.H.Q., Dubai, United Arab Emirates*
[2] *Biology Department, College of Science, UAE University, Al Ain, United Arab Emirates*

r.alghafri@hotmail.co.uk

Rapidly Mutating Y-STRs were proven to have significantly higher resolution in worldwide populations when compared to commonly used Y-STR kits [1]. In order to contribute to the researches that have been conducted, RM Y-STRs were investigated in parallel with Yfiler® kit in 327 male individuals from United Arab Emirates population. Such population is considerably isolated where Yfiler® and Powerplex® Y haplotypes were found to be shared between distantly related as well as non-related male individuals. In the present study, a comparison between Y-STR markers included in Yfiler® kit (DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385, DYS393, DYS391, DYS439, DYS635, DYS392, YGATAH4, DYS437, DYS438, DYS448) with RM Y-STRs (DYF387S1, DYF399S1, DYF403S1, DYF404S1, DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627) is conducted. RM Y-STRs were analysed using previously published methods [1]. Forensic Parameters were calculated for each set of markers, including discrimination capacity, haplotype diversity and gene diversity, using Arlequin v3.5 software.

1. Ballantyne, K.N., Keerl, V., Wollstein, A., Choi, Y., Zuniga, S.B., Ralf, A., Vermeulen, M., de Knijff, P., & Kayser, M. 2012. A new future of forensic Y-chromosome analysis: Rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic Science International: Genetics, 6, (2) 208-218.

69. PRESENCE OF Y-CHROMOSOME SEQUENCES IN DNA OF YOUNG FEMALE VICTIM OF SEXUAL ASSAULT

Maciejewska A.[1], Lipska-Ziętkiewicz B. S.[2], Koczkowska M.[2], Chamier-Ciemińska J.[1], Limon J.[2], Pawłowski R.[1]

[1] *Department of Forensic Medicine, Medical University of Gdańsk, Gdańsk, Poland*
[2] *Department of Biology and Genetics, Medical University of Gdańsk, Gdańsk, Poland*

Richard@gumed.edu.pl

A thirteen-year-old girl was forced to oral sex by a man she knew. A private laboratory A performed DNA typing. The SGMPlus® profiling revealed the victim's profile only and the Yfiler® typing gave negative results for all samples except for an oral swab, which revealed a haplotype of an unknown man. Inconsistency between DNA profiling results and victim's testimony resulted in the decision of the court to retest evidence samples. All DNA extracts were

retested in our laboratory and gave identical amplification signals for 9 of 23 Y-STR loci tested. Identified haplotype did not match either the suspect or an unknown man identified at laboratory A. DNA testing of the victim reference sample revealed the same Y-STR profile as obtained for all victim's swabs and lack of Y allele when amelogenin locus was analyzed. Analysis of X-chromosome specific loci identified some heterozygotes indicating the presence of two X chromosomes. Finally victim's DNA was subjected to the array-CGH testing, which revealed the presence of gains at chromosomal locations Yp11.31 and Yp11.2. Because of the lack of good quality DNA to perform karyotyping and FISH, we can only speculate whether the observed aberration results from a translocation of Y chromosome fragment to other chromosome or from the presence of a structurally abnormal Y chromosome. This case and previous observations of mutations at amelogenin gene strongly indicates that Y-STR typing of reference samples of female victims of sexual assaults should be performed.

70. MITOCHONDRIAL DNA ANALYSIS FOR PREDICTION OF RELATEDNESS AMONG VICTIMS OF SECOND WORLD WAR

Szabolcsi Z.[1], Dudas E.[2], Susa E.[2], Barany G.[1], Heinrich A.[1], Volgyi A.[1], Pamjav H.[1]

[1] *DNA Laboratory, Institute of Forensic Medicine, Network of Forensic Science Institutes, Budapest, Hungary*
[2] *Anthropology Laboratory, Institute of Forensic Medicine, Network of Forensic Science Institutes, Budapest, Hungary*

szabzoltan@gmail.com

During the reconstruction of Margaret Bridge human bone remains were found in the River Danube in Budapest, 2011. Due to underwater location of the remains, we supposed that these people might have got into the river together in the same period of time, likely at the end of the Second World War. Classical anthropological methods were used to predict gender and age of the deceased. The bones showed similar metrical and morphological characters. According to these results the remains might be originated from a population consisting of related peoples. DNA was isolated from 15 left femurs using EZ1 DNA Investigator Kit and EZ1 Advanced instrument (Qiagen®). Hypervariable regions of the mtDNA control region (HV1, HV2 and HV3) were amplified by Qiagen® Multiplex PCR Kit in different monoplex reactions.

F15971/H16401/L48/H408/F403/R599 primers were used in HV1-HV2-HV3 PCR and BigDye sequencing reactions. Haplogroups were predicted using haplogroup apomorphic control region SNPs originated from PhyloTree database and the tested sequences were aligned with known haplogroup sequences within the Mitosearch and FTDNA databases. Median Joining (MJ) networks within haplogroups were constructed to identify relationships of maternal lineages between the tested and database samples. Our results support the hypothesis that the bone remains belonged to related peoples.

71. MTDNA ROLE IN MIXTURES DECONVOLUTION

Omedei M.[2], Amayeh G. S.[1], Gino S.[1]

[1] *Department of Public Health Sciences and Pediatrics, University of Turin, Turin, Italy*
[2] *University of Rome "Tor Vergata", Department of Public Health, Rome, Italy*

mo.omedei@gmail.com

Mitochondrial DNA (mtDNA) usually has a negligible role in forensic caseworks and is taken into account by forensic scientists in case a familiar testing is necessary or when genomic DNA (gDNA) is degraded. No validated protocols to calculate the amount of mtDNA in a trace are normally implemented in the forensic workflow neither the ratio between gDNA and mtDNA is considered, with the exception of pure research approaches. Because of the great inter-individual and tissue-specific variability of mitochondria amount, the absence of this information may create misunderstanding in results interpretation. The aim of this work was to study mtDNA behaviour in mixtures composed of blood-saliva, blood-skin and saliva-skin; the GEP-ISFG group made similar analyses in the past, but only mixtures with semen were involved (representative of

rape samples, where a differential lysis was conducted) and the quite totally absence of mtDNA was observed in sperm fractions. In this project mixtures were prepared using two donor subjects with formerly known sequence information, having at least three different variants on the HV1 sequence, and a complete DNA extraction was carry out on all samples. Autosomal STR markers were also analyzed in order to compare sequence results with gDNA electropherograms. In our experiment it is considered for the first time the skin tissue as a DNA source in mixture where the role of mitochondria is pivotal. These results could represent the first step of a more wide analysis involving real-time detection of mtDNA, in order to understand real gDNA/mtDNA ratios in different contexts.

1. Montesino M, et al.: Analysis of body fluid mixtures by mtDNA sequencing: an inter-laboratory study of the GEP-ISFG working group. Forensic Science International. 2007; (168) 42-56.
2. Anslinger KD, Bayer BR, Rolf B, Eisenmenger W: mtDNA investigations after differential lysis. Journal of Forensic Sciences. 2005; (50)3: 579-581.
3. Crespillo M, et al.: Results of the 2003-2004 GEP-ISFG collaborative study on mitochondrial DNA: Focus on the mtDNA profile of a mixed semen-saliva stain. Forensic Science International. 2006; (160) 157-167.

72. IMPROVED RESOLUTION OF MITOCHONDRIAL MACROHAPLOGROUP M IN ABORIGINAL AUSTRALIANS; EVIDENCE FOR A NOVEL, AND ANCIENT M42 HAPLOGROUP

Nagle N.[1], van Oven M.[2], Ballantyne K. N.[3], van Oorschot R. A. H.[3], Wilcox S.[4], Turakulov R.[4], van Holst Pellekaan S.[5], McAllister P.[6], Williams L.[7], Kayser M.[2], Mitchell R. J.[1], The Genographic Consortium

[1] La Trobe University, Melbourne, Victoria, Australia
[2] Department of Forensic Molecular Biology, Erasmus University Medical Center Rotterdam, The Netherlands
[3] Office of the Chief Forensic Scientist, Victorian Police Forensic Services Department, Melbourne, Victoria, Australia
[4] Australian Genome Research Facility, Melbourne, Victoria, Australia
[5] University of New South Wales, Sydney, Australia
[6] Griffith University, Queensland, Australia
[7] Department of Communities, Child Safety and Disability Services, Queensland Government, Queensland, Australia

n.nagle@latrobe.edu.au

Australian Aboriginal people have one of the oldest continuous cultures outside Africa, with their ancestors arriving in the ancient landmass of Sahul (present day Papua New Guinea (PNG) and Australia) ~50 thousand years ago (KYA). Genetic studies, though small in scale, have demonstrated both the uniqueness and antiquity of Australian Aboriginal Y and mitochondrial (mtDNA) lineages.

Females carrying both mtDNA macrohaplogroup M and N lineages entered Australia at least 50 KYA, as evident by the presence of Australian specific haplogroups; M42a, N13, S, O and some P subtypes. Haplogroup M42a is of particular interest because of its deep phylogenetic association with the Indian haplogroup, M42b. The presence of haplogroup Q is also of interest, given its presence in PNG and Melanesia.

We examined mt SNPs, the hypervariable region (HVR) and whole genomes among 500 Aboriginal Australians, with particular focus on macrohaplogroup M diversity, from locations previously either poorly or never sampled.

The M42a lineage was widespread across the continent and we identified novel sublineages. More significantly we have identified a novel M42 subtype, provisionally labelled M42c. This haplogroup was also found to be frequent, widespread and could also be characterised into sublineages. Estimates of Time to Most Recent Common Ancestor (TMRCA) of both M42a and M42c give values greater than 40 KY and indicate that M42c is ~10 KY older than M42a. M42c is, like M42a, an Aboriginal Australian specific haplogroup.

73. NEW INSIGHTS INTO THE ARAWAKAN DIASPORA: MTDNA CONTROL REGION ANALYSIS OF TWO POPULATIONS

Xavier C.[1], Bodner M.[1], Nagl S.[1], Huber G.[1], Silva D. A.[2], Casas-Vargas A.[3], Usaquén W.[3], Gusmão L.[2,4], Parson W.[1,5]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[2] *DNA Diagnostic Laboratory (DDL), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil*
[3] *Group of Population Genetics and Identification, Genetics Institute, National University of Colombia, Bogotá, Colombia*
[4] *Institute of Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal*
[5] *Forensic Science Program, The Pennsylvania State University, PA, USA*

catarina.gomes@i-med.ac.at

Among South American linguistic families, the Arawakan are currently described as one of the most widespread languages in the continent, occupying a very broad and diverse territory from Southern Brazil to the Caribbean Islands. The Arawakan groups are thought to owe their dispersal success to their agricultural habits, which have allowed an increase in population density, as well as the advent of more complex societies on cultural, religious and inter-population social practices. Aiming to shed further light on the maternal lineages of the Arawakan, we analyzed the full control region of the mitochondrial DNA from Arawakan speaking groups. Here we present the results of two populations inhabiting different edges of the Arawakan diaspora, namely the Terena from Southern Brazil and the Wayuu from La Guajira peninsula in Northern Colombia. Our results describe highly diverse groups that display different Pan-American mtDNA haplogroup distributions, pinpointing effects of geographical distance rather than their linguistic relation. The advances in description of maternal genetic lineages of extant and isolated populations are highly relevant to further unveil South America's initial colonization panorama. Furthermore, this characterization is of extreme importance to boost quality, accuracy and size of forensic mtDNA databases, especially in highly structured groups as Native American populations.

74. THE BENEFITS AND LIMITATIONS OF EXPANDED Y-STR LOCI

Simon C.[1,2], Henry J.[1,2], Linacre A.[1]

[1] *Flinders University of South Australia, Adelaide, Australia*
[2] *Forensic Science SA, Adelaide, Australia*

julianne.henry@sa.gov.au

Y-STR analysis using the Life Technologies 17 locus Yfiler® kit was introduced at Forensic Science SA (FSSA) in 2007. This kit has been used widely in casework and has provided probative information in many cases. Life Technologies recently released its 27 locus Yfiler® Plus kit to the market. This kit has complete overlap with the 17 loci contained in Yfiler® with the addition of 10 rapidly mutating (RM) Y-STRs.

FSSA has evaluated the Yfiler® Plus kit for improved sensitivity, discrimination power and overall performance. Studies to date have shown an increased sensitivity of up to 20-fold over Yfiler® and an increased discrimination power of paternally-related males. Yfiler® Plus also demonstrated good performance in regard to detection of male DNA in mixed samples and amplification of mock trace DNA samples. Investigation of the kit using FSSA casework samples is currently underway.

While it is likely that FSSA will implement Yfiler® Plus into routine casework, the inclusionary probability of any individual is unlikely to increase significantly despite the increased number of loci. Other issues such as interpretation of expanded loci, generation of relevant local haplotype databases to include the expanded haplotype, and increased mutations between closely related males where proof of relatedness is required need to be addressed. The benefits and limitations of this kit will be discussed in more detail.

75. ANALYSIS OF HIGH MUTABLE Y-STRS IN URBAN AND AMERINDIAN ARGENTINEAN POPULATIONS

Bobillo C.[1,2], Sala A.[1,2], Corach D.[1,2]

[1] *Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry Universidad de Buenos Aires, Argentina*
[2] *Consejo Nacional de Investigaciones Científicas y Tecnológicas-CONICET, Argentina*

shdg@ffyb.uba.ar

The Y-chromosome STR analysis has been useful in establishing patterns of human migrations through the characterization of paternal lineages and has also been used for forensic and anthropological purposes. However these markers exhibit a limitation concerning its mutation rates. On one hand, recurrent mutations lead to observe individuals with identical haplotypes without belonging to the same patrilineage, and on the other, low mutation rates make difficult to differentiate between haplotypes that share the same Y-chromosome linage.

Recently, a battery of Y-STRs with a very high mutation rate has been developed, called 'rapidly mutating (RM) Y-STRs', their main feature allows focusing their use under a new perspective in forensics. In order to evaluate their forensic parameters we analyzed two population groups from Argentina: an urban (N=297) population and a set of samples (N=175) belonging to different Native American groups.

The results showed that the average gene diversity (GD) was higher in urban populations (0,8471) compared with the isolated groups (0,8322). Moreover, haplotype sharing within populations was highest in Amerindian groups (2,85%, 5 haplotypes shared) versus urban population (0,6%, 2 haplotypes shared). Interestingly, shared haplotypes were not observed between the different tribal groups. The results between and within urban and Amerindian groups are consistent with previous studies. The percentage of shared haplotypes within the Amerindian group could be explained by a higher endogamous behavior that characterizes these isolated and marginal populations. Development of local RM-YSTR reference databases is recommended when forensic evidentiary material samples might be obtained from isolated groups.

76. THE FINDING OF Y-STR MICRODELETION INVOLVING DYS448, DYS392, DYS549 AND DY385A/B MARKERS IN A PATERNITY CASE WITH DECEASED ALLEGED FATHER

Beltramo J.[1], Pena M. A.[1], Lojo M. M.[1]

[1] *Laboratorio de Análisis Comparativo de ADN. Suprema Corte de Justicia de la provincia de Buenos Aires, Argentina.*

mercedeslojo@hotmail.com

In the context of a post mortem paternity case with the alleged grandfather as the only available reference sample, an acceptable LR value was reached (363830) with the profiles of 20 autosomal markers (PowerPlex21, Promega). The child's Yfiler (Applied Biosystems) haplotype showed a signal loss affecting markers DYS385a/b, DYS392 and DYS448. This finding was confirmed in the YPlex23 (Promega) profile where the loss of signal was also observed in DYS549 marker. These results taken together are compatible with a micro deletion affecting the long arm of the Y chromosome, in the AZFb+c deletion region.

Since the grandfather's haplotype showed a complete profile, it could be assumed that the observed micro deletion is a de novo event which could have been occurred during grandfather's or father's meiosis. In addition, an inconsistency between Grandfather/child profile was also observed in the DYS481 marker, which could be explained by assuming a meiosis mutation (23 to 24).

Although the autosomal markers support the biological relationship between grandfather and child, the lost of information due to the chromosomal deletion

besides the inconsistency detected in the DYS481 marker badly affects the LR value (LR = 145, estimated with the kinship tool, https://yhrd.org/kinship/check).

77. Y STRS MUTATION EVENTS IN FATHER-SON PAIRS IN ECUADORIAN INDIVIDUALS

Sánchez M. E.[1], Burgos G.[1], Gaviria A.[2], Aguirre V.[2], Vela M.[2], Zambrano A. K.[2], Leone P. E.[1], Paz-y-Miño C.[1]

[1] *Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito – Ecuador*
[2] *Laboratorio de Genética Molecular, Cruz Vital - Cruz Roja Ecuatoriana, Quito – Ecuador*

anibalgaviria@hotmail.com

Few Y chromosome studies still exist in Ecuadorian populations that allow their genetic characterization, therefore our study pretends to incorporate new results about mutation rates at Y chromosome information. Eleven Y-STR loci (included in PowerPlex Y System®) was used to genotype 206 confirmed father-son pairs. Based in this sample, mutation rate was estimated, and prediction of haplogroup was obtained for all of them. Seven mutations that comprise single step changes were observed in 2532 allelic transfers, locus involved were DYS385, DYS390, DYS391, DYS392 and DYS389II. Sample mutation rate estimated in this study was 2.8 x 10-3 (95% CI, 1.1 x 10^{-3} to 5.7 x 10^{-3}); this result agrees with reports in other populations[1, 2]. Nevertheless, although frequency for Q haplogroup was 37.6%[3], mutation rate was higher in this lineage than the others (5 to 2). These results will contribute to forensic, paternity and evolution areas within the analysis of genetic population of Ecuador.

1. Ge J., Budowle B., Aranda X.G., Planz J.V., Eisenberg A.J., Chakraborty R. 2009. Mutation rates at Y chromosome short tandem repeats in Texas population. Forensic Sci. Int. Genet. 3: 179-184
2. Gusmão L., Sánchez-Diz P., Calafell F., et al: Mutation rates at Y chromosome specific microsatellites. Hum. Mutat. 2005, 26: 520-528
3. Gaviria A, Sánchez ME, Morejón G et al: Characterization and Haplotype analysis of 11 Y-STR loci in Ecuadorian population. Forensic Sci. Int. 2013; 4: e310-e311

78. GENETIC ANALYSIS OF Y-CHROMOSOMAL STR OF SIX POPULATIONS IN ASIA

Lee J. H.[1], Cho S.[1], Min H. W.[1], Lyoo S. H.[1], Lee S.[1,2]

[1] *Department of Forensic Medicine, Seoul National University College of Medicine, Seoul, Korea*
[2] *Institute of Forensic Medicine, Seoul National University College of Medicine, Seoul, Korea*

jh714@snu.ac.kr

The human population in Asia is characterized by migration events of mankind, as well as admixture with Europeans. Detailed information derived from Y chromosomal STR might provide further information on personal identification or phylogenetic history. However, the data is not sufficient for Asian populations. In this study, we investigated the genetic diversity of the 23 Y-chromosomal short tandem repeat (Y-STR) loci and typed in 298 unrelated males, who are not in YHRD, residing in southern China(n=49), Beijing(n=50), Japan(n=61), Vietnam(n=46), Nepal(n=69) and India(n=23) using PowerPlex® Y 23 System. A total of 298 haplotypes were identified and analyzed using AMOVA. The results showed that when 6 ethnic groups were compared, Population pairwise FSTs were found to be less than 10%. In order to examine the genetic relationship between the Korean and other populations, data of 300 Korean males from YHRD were added. Nevertheless the genetic differentiation between Beijing and the Japanese was very low, these pairwise comparisons revealed significant differences between south Asia population (India and Nepal) and the others. Genetic uniqueness of the examined groups in this study will support in the creation of databases for population genetic studies. Larger sample sizes will be necessary to fully understand the

population structure and to discriminate them in details.

1. P De Knijff, M Kayser, D Corach, et al: Chromosome Y microsatellites: population genetic and evolutionary aspects. International Journal of Legal Medicine. 1997; 110(3): 134-140.
2. S Willuweit and L Roewer: The new Y Chromosome Haplotype Reference Database. Forensic Science International: Genetics. 2015; 15: 43-48.

79. MASSIVELY PARALLEL SEQUENCING OF MITOCHONDRIAL DNA CONTROL REGION AND SNPS FOR GLOBAL HAPLOGROUP DETERMINATION

Lee E. Y., Jung S. E., YangI. S., Shin K. J.

Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

kjshin@yuhs.ac

Because of the potential of high-throughput sequencing for recovering genetic information from multiple markers and multiple individuals in a single run, forensic genetic application of next generation sequencing (NGS) technology has been explored by increasing number of laboratories. However, NGS analysis of mitochondrial DNA (mtDNA) requires cumbersome and technically challenging library construction process. Therefore, we developed a simplified library preparation method which comprises only 2 rounds of PCR. In the first-round of multiplex PCR, 6 fragments targeted for the entire control region and 22 fragments targeted for many interspersed coding region SNPs designating global haplogroups and East Asian haplogroups were amplified using template-specific primers with read sequences. Then, in the following step, indices and platform-specific sequences for the MiSeq® System (Illumina) were added by PCR. The barcoded library produced by these simplified workflows could be successfully sequenced on a MiSeq using MiSeq Reagent Nano Kit v2. A total of 0.4 GB sequences with 80.6% of >Q30 from 12 degraded DNA samples were obtained and finally mapped to the rCRS. The average coverage was about 5,700×, and relatively even read count was obtained to have less than three times of read count difference in a sample. Control region sequences were determined and relevant haplogroups were assigned for all the samples. Moreover, since the multiplex PCR system comprises small amplicons less than 250 bp, NGS analysis with the developed library preparation method will facilitate the mtDNA analysis even from highly degraded DNA samples.

80. CHOOSING ANALYTICAL THRESHOLDS FOR YFILER PLUS DYES

Andersen M. M.[1], Mogensen H. S.[2], Eriksen P. S.[1], Morling N.[2]

[1] Department of Mathematical Sciences, Aalborg University, Aalborg, Denmark
[2] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

mikl@math.aau.dk

In forensic genetic STR analysis, the analytical threshold affects the balance between drop-outs of true alleles and drop-ins of false alleles. Most STR analysis softwares do not allow for floating or sample dependent thresholds. Therefore, average thresholds for all alleles are commonly used. The introduction of the AB3500/AB3500xl Genetic Analyzers (Life Technologies) has led to the need for dye dependent thresholds, since the signal intensities vary between the fluorescence dyes. In this study we used the Yfiler® Plus DNA Amplification Kit (Life Technologies) that has five fluorescent dyes incorporated in the PCR primers to investigate the analytical thresholds both in reference samples and in dilution series of known DNA. We analysed the peak heights of the samples with the purpose of investigating how to choose dye dependent analytical thresholds. We compared the results with those of previously published methods for modelling known phenomena in STR typing such as dropouts. We used receiver operating characteristic (ROC) curves to choose the analytical thresholds weighing the sensitivity and specificity. We also considered dropout probabilities modelled by a logistic regression model using a proxy of the signal strength as predictor. Two signal strength proxies were investigated:

(1) the mean peak height of alleles not dropped out and (2) a more advanced proxy including the degree of the locus imbalance.

81. EVALUATION OF MTDNA STABILITY ACROSS THE MATERNAL LINE: A STUDY ON THREE GENERATIONS

Cortellini V., Verzeletti A., Cerri N., Cisana S., De Ferrari F.

University of Brescia – Department of Medical and Surgical Specialties, Radiological Sciences and Public Health - Forensic Medicine Unit - Brescia, Italy

venusia.cortellini@unibs.it

Sometimes deficient paternity testing can't be resolved with routine STR analysis and nuclear DNA fails to give conclusive results. At these cases, mitochondrial DNA (mtDNA) analyses have been proved more effective. Indeed, excluding mutations, mtDNA sequence is identical for all maternally linked relatives. The feature of maternal inheritance can be useful to support or refute the identity of samples by comparison with reference samples from known maternal relatives. In general, mtDNA transmission is stable across many generations, therefore the mtDNA typing allows to assess maternal relationships, even in deficient parenthood. To evaluate the consistency of mtDNA, we compared HVI and HVII regions in three generations along the maternal line (great-grandmother, grandmother, mother, daughter). STRs profile was also defined for each individual. Preliminary results don't show haplotype dissimilarities, confirming the useful of this marker in such cases.

1. Budowle B, Allard M.W., Wilson M.R., Chakraborty R.: Forensics and Mitochondrial DNA: Applications, Debates, and Foundations. Annu Rev Genomics Hum Genet. 2003; 4:119–41.
2. Lutz S., Weisser H.J., Heizmann J., Pollak S.: mtDNA as a tool for identification of human remains. Int J Legal Med. 1996; 109:205-9.
3. Sullivan KM, Hopgood R, Gill P: Identification of human remains by amplification and automated sequencing of mitochondrial DNA. Int J Legal Med. 1992; 105: 83-6.

82. MITOCHONDRIAL DNA DIRECT PCR SEQUENCING OF BLOOD FTA PAPER

Nogueira T. L. S.[1,2], Oliveira T. P.[1,2], Braz E. B. V.[1], Santos O. C. L.[1], Silva D. A.[2], Carvalho E. F.[2]

[1] Instituto de Biologia do Exército, Rio de Janeiro, Brazil
[2] Laboratório de Diagnósticos por DNA, DECOL, IBRAG, Universidade do Estado do Rio de Janeiro, Brazil

elizeufc@hotmail.com

In the forensic context, the mtDNA analysis represents a powerful methodology, particularly for highly degraded DNA, since the collection and storage of mitochondrial genetic profiles can add important information to various applications in human and other species identification. Several sources of human DNA may be evaluated by different sampling procedures. In this study, we analyzed the control region of mtDNA of long-term storage reference samples. The aim of this study was to improve the mtDNA sequencing from samples collected on FTA paper. We have used dried blood spots of 21 militaries from the Brazilian Army Repository of Samples. In order to do this, two discs of 1.2 mm were taken from each blood sample and washed with a buffer containing Proteinase K. Afterwards, they were transferred to a tube containing 10µL of PCR amplification reaction. The PCR amplification products have been purified in order to running the mtDNA sequencing protocols which were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The unincorporated dye terminators were removed using the DyeEx 2.0 Spin Kit (Qiagen) according to the manufacturer's instruction. Capillary electrophoresis was carried out on an ABI 3500 Genetic Analyzer (Applied Biosystems) and the generated data analyzed using the DNA Sequencing Analysis software v5.2 (Applied Biosystems). We have shown that the Direct mtDNA sequencing technique can be optimized by using a rapid and low-cost method without the extraction DNA step and the purification

step after PCR amplification. Samples were successfully performed generating satisfactory and reproducible results, which makes this methodology applicable for large-scale analysis.

1. Grujičić NK, Davidović S, Takić D, Mojsin M and Stevanović M. Direct PCR amplification of the HVSI region in mitochondrial DNA from buccal cell swabs. Arch. Biol. Sci. 2012, 64 (3), 851-858.
2. Holland MM; Parsons TJ. Mitochondrial DNA sequence analysis validation and use for forensic case-work. Forensic Science Review. 1999, v. 11, n. 1, p. 21-50.
3. Kareem MA1, Abdulzahra AI, Hameed IH, Jebor MA. A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. Mitochondrial DNA. 2015, v.23, p. 1-5.

83. CHARACTERIZATION OF THE STR LOCI ALLELE'S DISTRIBUTION OF Y CHROMOSOME WITH HIGH MUTATION RATE IN POPULATION SAMPLE OF RIO DE JANEIRO

Jannuzzi J.[1], Silva D. A.[1], Carvalho E. F.[1]

[1] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil

elizeufc@hotmail.com

Y chromosome genetic markers are used for characterization of male lineages, since they are fully transmitted to next generations unless mutations occurs [1,2,3]. Y-STR Markers are widely applied in forensic genetics because of their high capacity of discriminate lineages. The 13 rapidly mutating newly discovered Y-STR markers (RM-YSTR) show highly mutation rates in comparison to other common Y-STRs [4,5]. As RM-YSTRs display high mutation rates and efficiency in discriminating paternally related males, we aimed to deepen the knowledge about population and mutational RM-YSTR loci characteristics in Rio de Janeiro sample, which is known to be mixed. RM-YSTR analysis was realized in 258 males born in Rio de Janeiro state, grouped in 129 fathers/sons pairs. RM-YSTR loci showed a discrimination power of 1.0 (±1.2190), low haplotype frequencies (0.0077) and haplotype diversity of 1.0 (±1.2190). Moreover, high values of genetic diversities were obtained for the 13 markers. These results are associated with high mutation rates found, with an average rate about 2.11×10^{-2}. In 28 fathers/sons pairs were observed 30 mutations over RM-YSTR loci, excepting DYS518 and DYS626 loci. All 30 were single-step mutations, showing an excess of 18 repeat gains over 12 repeat losses. Due to their high mutation rates and discrimination capacity, RM-YSTR showed to be very discriminative at this mixed sample, besides proving to be more discriminative than other markers commonly used in population studies and forensic analysis.

1. Singh N.P., et al. Epigenetic profile of the euchromatic region of human Y chromosome. *Nucleic Acids Res* 2011, 39 (9): 3594-3606 p.
2. Mitchell R.J.; Hammer M.F. Human evolution and the Y chromosome. *Curr Opin Genet Dev* 1996, 6 (6): 737-742 p.
3. Butler J.M. *Fundamentals of forensic* DNA typing: Academic Press. 2009
4. Ballantyne K.N., et al. Mutability of Y-chromosomal microsatellites: rates, characteristics, molecular bases, and forensic implications. *Am J Hum Genet* 2010, 87 (3): 341-353 p.
5. Ballantyne K.N., et al. Toward male individualization with rapidly mutating y-chromosomal short tandem repeats. *Hum Mutat* 2014, 35 (8): 1021-1032 p.

84. PHYLOTREE BUILD 17: GROWING THE HUMAN MTDNA TREE

van Oven M.

Dr. Molewaterplein 50 3000 CA Rotterdam, Netherlands

mannis@gmail.com

An accurate mtDNA phylogenetic tree is essential in forensics for mtDNA data quality control, for estimating site-specific mutation rates, and for haplogroup assignment allowing matrilineal biogeographic ancestry inference. In 2008, a comprehensive worldwide mtDNA phylogeny was published [1]. During subsequent years, regular updates of the initial tree have been released through the PhyloTree website (http://www.phylotree.org), bringing its resolution from a total of 1267 defined branches in Build 1 to a total of 4809 branches in Build 16. PhyloTree has been widely adopted in the forensic genetics community, as well as in the fields of mitochondrial medicine, human population genetics, and genetic genealogy. It serves as the underlying haplogroup classification system for EMPOP [2], HaploGrep [3] and Phy-Mer [4], among others [5]. This poster introduces the newest version of PhyloTree, Build 17, and describes its features.

1. van Oven M., Kayser M.: Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009; 30: E386 – 94
2. Röck A.W., Dür A., van Oven M., Parson W.: Concept for estimating mitochondrial DNA haplogroups using a maximum likelihood approach (EMMA). Forensic Sci Int Genet. 2013; 7: 601 – 9
3. Kloss-Brandstätter A., Pacher D., Schönherr S., Weissensteiner H., Binna R., Specht G., Kronenberg F.: HaploGrep: a fast and reliable algorithm for automatic classification of mitochondrial DNA haplogroups. Hum Mutat. 2011; 32: 25 – 32
4. Navarro-Gomez D., Leipzig J., Shen L., Lott M., Stassen A.P., Wallace D.C., Wiggs J.L., Falk M.J., van Oven M., Gai X.: Phy-Mer: a novel alignment-free and reference-independent mitochondrial haplogroup classifier. Bioinformatics. 2014; in press
5. Bandelt HJ, van Oven M, Salas A: Haplogrouping mitochondrial DNA sequences in Legal Medicine/Forensic Genetics. Int J Legal Med. 2012; 126: 901 – 16

85. COSEGREGATION ANALYSES OF Y CHROMOSOMES AND SPANISH SURNAMES REVEAL FEATURES OF INTEREST TO POLICE INVESTIGATIONS

Martinez-Cadenas C.[1], Blanco-Verea A.[2], Hernando B.[1], Busby G. J. B.[3], Carracedo Á.[2,4], Brion M.[2], Salas A.[4], Capelli C.[3]

[1] Department of Medicine. Jaume I University of Castellon. Castellon, Spain
[2] Instituto de Investigación Sanitaria de Santiago de Compostela, Grupo de Medicina Xenómica, Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain
[3] Human Evolutionary Genetics Group. Department of Zoology University of Oxford. Oxford, United Kingdom
[4] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, Spain

ccadenas@uji.es

In most human societies, surnames are passed from fathers to children, just like the Y-chromosome. So, theoretically, men sharing the same surnames should also share similar Y-chromosomes. In this study, the aims were to determine the correlation between surnames and Y-chromosomes in Spain, to calculate the TMRCA for haplotypes belonging to the same surname that cluster together (descent clusters), and to assess the effect of geography in the degree of coancestry within surnames.

A total of 1,766 DNA samples from male volunteers belonging to 37 Spanish surnames plus 355 controls were collected and genotyped for Y-chromosome SNPs and STRs.

We found that the degree of coancestry of men bearing a rare surname was quite high, while it was extremely low for frequent surnames. That is, coancestry was highly dependent on surname frequency. However, we demonstrate that the effect of geography is crucial, since enrichment of surnames with the collection of additional men born in the same region significantly increases

coancestry within most surnames.

Besides, TMRCAs were calculated for all descent clusters, with most time depths falling within the time of surname establishment in Spain (about 500-800 years ago).

Studies like the present one, or previous similar studies undertaken in the British Isles, not only shed light on population structure and history, but also suggest a link between particular Y-chromosome lineages and surnames. This link may be potentially helpful in police investigations as, in the future, Y-chromosome profiles could be used to predict surnames in certain circumstances.

86. MITOCHONDRIAL DNA TYPING OF LASER-CAPTURED SINGLE SPERM CELLS TO DIFFERENTIATE INDIVIDUALS IN A MIXED SEMINAL STAIN

Zhang L.

Department of Forensic Medicine, National Police University of China, No. 83, Tawan Street, Huanggu District, Shenyang 110854, China

zhangluzhaoyang@sohu.com

The identification of donors in a mixture of two semen samples usually involves a combined analysis of autosomal and Y chromosomal short tandem repeats (STR) genotyping, which can exclude unrelated individuals but cannot achieve the purpose of personal identification. Since multiple-copy mitochondrial DNAs (mtDNA) exist in sperm cells and exhibit genetic polymorphisms in different matrilineal individuals, we can apply laser-captured technology[1] to perform nested polymerase chain reaction (PCR) on the mtDNA hypervariable region I (HV I) of single sperm cells in the mixed sample. Then amplicons of mtDNA were sequenced by Sanger method, and each extracted DNA sample with the same HV1 sequence was collected and genotyped by Promega PowerPlex 16HS kit. Also, sexual assault casework involved two male donors was examined by our method. The results showed the mtDNA sequence analysis can be used to enrich the sperm cell DNA with the identified sequence and autosomal STR from cell nuclei can be genotyped successfully, thereby identification on different individuals can be achieved in the mixed semen sample.

1. Li C.X., Han J.P., Ren W.Y., Ji A.Q., Xu X.L. and Hu L.: Profiling of Spermatozoa by Laster Capture Microdissection and Low Volume-PCR. PLOS ONE. 2011; 6: e22316.

87. DIFFERENTIATING BETWEEN MONOZYGOTIC TWINS IN FORENSICS THROUGH NEXT GENERATION MTGENOME SEQUENCING

Wang Z., Zhang S., Bian Y., Li C.

Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science, Ministry of Justice, P.R. China, Shanghai, China

wangzhengtim@163.com

Monozygotic (MZ) twins, considered being genetically identical, cannot be distinguished one from another by forensic short tandem repeat (STR) profiling. The high mutation rate of the mitochondrial DNA (mtDNA) has the potential to become a promising biomarker for the differentiation between MZ twins. With the advent of Next-Generation Sequencing (NGS) approaches, it is now possible to characterize minor differences of mtDNA genomes (mtGenomes) between MZ twins. In this study, we mapped nucleotide differences and heteroplasmies of MZ twins' mtGenomes by NGS technology. Blood samples were taken from 6 pairs of adult MZ twins, and the mtGenomes were sequenced using the Illumina HiSeq 2000 Sequencing System. The average coverage reached 40,000 × and bioinformatics analysis revealed that an average of 3-5 bases present varying degrees of heterogeneity between mtGenomes of MZ twins and a single nucleotide difference (e.g. nt15301, 96.88% G-reads in MZ twin A and 88.09% A-reads in MZ twin B) exists in four pairs of MZ twins. Our results give experimental evidence for the hypothesis that variants of mtGenomes

could be a perspective biomarker to distinguish MZ twins from each other.

88. THE MITOCHONDRIAL DNA POLYMORPHISMS IN CHROMOSOMAL ABERRATION DETECTED BY NGS

Honda K., Iwabuchi Y., Yano S., Sugano U.

Department of Legal Medicine , University of Tsukuba, 305-8575, Japan

k-honda@md.tsukuba.ac.jp

Total mtDNA genome in Turner`s and Down`s syndrome were analyzed. Long range PCR were performed with Nextera preparation spanning the entire human mitochondrial genome (16,569 bp). Library sequencing on the MiSeq (Illunnina) is followed by data analysis with with the mtDNA variant analyzer. Blood samples were collected from the case of sudden death of Down`s syndrome and Turner`s (including mother and affected daughter). The analytical procedure were carried out following instructions of „Human mtDNA Genome" for Illumina Sequencing Platform. Mt DNA sequence in Turner`s female was found to be unique, and specific, as it was not found in the normal population. The mtDNA from Down's syndrome and Turner`s syndrome was found to have shared at least seven position of mutation sites. Astonishingly, family data exhibited that the haplotype of the mtDNA in the daughter was apparently different from the mother's, suggesting that the mtDNA of Turner female would not be inherited from mother to daughter. Our data indicate that possible interaction of the sex chromosome and the mtDNA exists. Also, it was suggested that mitochondrial mutation was not unrelated to the chromosomal aberrations. We already reported that specific polymorphism and maternal hereditary collapse of mitochondrial DNA in Klinefelter's syndrome[1)]. We suspected that the specific mtDNA haplotype could cause the abnormal cell to fertilize and reconstruct after fertilization.

1. Oikawa H, Tun Z, Honda K: The specific mitochondrial DNA polymorphism found in Klinefelter's syndrome. BBRC .2002, 297: 341–345

89. FULL MT-GENOME SEQUENCING WITH MITOCHONDRIAL TILING PATH PRIMERS USING THE ION TORRENT PGM

Strobl C.[1], Lagace R.[2], Chang J.[2], Parson W.[1,3]

[1] Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria
[2] Thermo Fisher Scientific Inc., South San Francisco, CA, USA
[3] Forensic Science Program, The Pennsylvania State University, PA, USA

Christina.Strobl@i-med.ac.at

For forensic applications the analysis of mitochondrial DNA (mtDNA) is a meaningful tool, especially for samples where nuclear DNA markers fail to give useful results. For these approaches Sanger sequencing of the non-coding control region (CR) is the current standard. It has been shown that sequencing of complete mitochondrial Genomes (mtGenomes) provide a significantly higher discrimination power compared to the analysis of the CR. This is true for both randomly selected population samples as well as most common CR types. We have further demonstrated that it is feasible to recover full mtGenome sequences from as little as the mtDNA amount extracted from 2 cm hair shaft samples, which cannot be achieved applying conventional Sanger technologies. This self-developed assay is using 62 midi-sized amplicons, 300 to 500 bp in size, targeted in two multiplex reactions consisting of 31 PCR products each. Meanwhile, a commercial product has been developed that also targets the entire mtGenome using 162 amplicons with about 175 bp in size. The smaller amplicon sizes would be useful for the amplification of heavily degraded DNA and thus suitable for challenging casework samples. Here, we report our findings using the new mito tiling path primer pools for difficult forensic samples including hair shafts and historic samples (bone/teeth).

90. PROOF-OF-PRINCIPLE-STUDY FOR THE INVESTIGATION OF SHORT MITOCHONDRIAL DNA FRAGMENTS USING NEXT GENERATION SEQUENCING

Zander J.[1], Hecht J.[2], Nagy M.[1]

[1] *Department of Forensic Genetics, Institute of Legal Medicine and Forensic Science, Charité-Universitätsmedizin, Berlin, Germany*
[2] *Berlin-Brandenburger Centrum für Regenerative Therapien, Charité–Universitätsmedizin, Berlin, Germany*

Judith.zander@charite.de

Latest technical improvements showed that Next Generation Sequencing (NGS) will be a reliable alternative to the conventional Sanger Sequencing method. By using this high-throughput-procedure millions of sequence reactions are processed simultaneously to reach an increased sensitivity and detectability. Therefore, the knowledge of the genome becomes more detailed.

Although the Next Generation Sequencing technology is still time-consuming and expensive, the high level of gain in perception cannot be ignored. This is of importance, especially with regard to ancient and degraded DNA, because such problematic samples can be successfully analysed with the help of Next Generation Sequencing.

In this presentation we want to illustrate preliminary NGS-analysis of short mitochondrial fragments. We proved the effort and benefit of this method by comparing the results with Sanger Sequencing and performed tests to detect the sensitivity and functionality. The gain in information is presented by means of problematic forensic traces.

91. POPULATION GENETICS OF 26 Y-STR LOCI IN SHE ETHNIC MINORITY FROM FUJIAN PROVINCE, SOUTHEAST CHINA

Bian Y.[1], Zhang S.[1], Wang Z.[1], Li C.[1]

[1] *Institute of Forensic Sciences, Ministry of Justice, P.R. China*

bianyn@ssfjd.cn

The She people, with a population of about 710,000, are the largest minority in Fujian province. They live primarily in the mountainous areas of Fujian Province and also present in the provinces of Zhejiang, Anhui, Jiangxi and Guangdong. Some descendants of the She also exist amongst the Hakka minority in Taiwan. Currently, forensic Y-STR analysis has well characterized and has become an essential tool in forensic genetics. Although it has been demonstrated that the 26plex Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385ab, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, GATA H4, DYS549, DYS643, DYS388, DYS570, DYS533, DYS576, DYS460, DYS481 and DYS449) typing system is reproducible, accurate, sensitive and robust in Chinese Han population, there was a lack of data for She population. In this study we evaluated 100 unrelated male individuals of Chinese She living in the Fujian Province to explore the underlying genetic structure of She populations. These samples were typed i by using the 26plex Y-STRs typing system. A total of 92 different haplotypes were found. Both Rst pairwise analyses and multidimensional scaling plot showed the genetic structure of She population was significantly different from some of Chinese Han populations. It revealed that the 26plex Y-STRs typing system provided substantially stronger discriminatory power in She population.

92. REGIONAL PATTERNS OF Y-CHROMOSOME VARIATION IN BOLIVIA

Cárdenas J. M.[1], Heinz T.[1], Pardo-Seco J.[1], Álvarez-Iglesias V.[1], Taboada-Echalar P.[1], Sánchez-Diz P.[1], Carracedo Á.[1], Salas A.[1]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain

antonio.salas@usc.es

The Bolivian population constitutes one of the most representative Native American countries in America, where the Native American community accounts for about 55% of the total population. Apart from Europeans, during colonial times Bolivia was also the destination of a number of Africans, who were forced to work at the Royal Mint in Potosí. Some 'Afro-Bolivian' communities exist today in the country but these communities received very little attention in the literature. In the present study a sample of 226 Bolivian males was recruited from three different eco-geographical regions (Andean, Sub-Andean and Llanos). We analyzed 32 Y-SNPs in order to determine their haplogroup adscription to main continental ancestries. Moreover, in order to evaluate patterns of Y-chromosome stratification and diversity, we additional genotyped the 17 Y-STRs included in the commercial Yfiler kit. Additionally, comparisons with autosomic AIMs and mitochondrial DNA data (mtDNA) were carried out to evaluate the level of continental admixture in the sample and the distribution of variation by gender. The results of these comparisons showed a typical gender-bias pattern in the Bolivian population characterized by a high European composition in the Bolivians Y chromosomes and a remarkable Native American composition in the mtDNA and in the autosomic markers. Furthermore, African profiles were also detected in two of the three regions summing-up 6% of total sample. Finally, Bolivian population showed high diversity indices for the Y-STRs. AMOVA analyses indicate that Bolivians contribute to a large amount of population stratification in South America when compared to other populations from this sub-continent.

93. EXAMINING PATTERN OF MITOCHONDRIAL DNA VARIATION IN CHILE, FROM NORTH TO THE MOST SOUTHERN CONE

Gómez-Carballa A.[1,2], Moreno F.[3], Álvarez-Iglesias V.[1], Martinón-Torres F.[2,4], Pantoja-Astudillo J. A.[3], Aguirre Morales E.[3], Bustos P.[3], Salas A.[1,2]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain
[2] Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain
[3] Servicio Medico Legal, Ministerio de Justicia, Santiago, Chile
[4] Infectious Diseases and Vaccines Unit, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain

antonio.salas@usc.es

Chile is particularly long and narrow, which combined with its mountainous nature and its complex past demography of admixture, provides a unique scenario for population genetic studies. Its official census does not contain information on ethnicity, whereas various studies consider that most Chileans can be 'ethnically' allocated to two main 'phenotypic' groups ('blancos' and 'mestizos'). Genetic studies are also scant in Chile. We have obtained 995 control region mitochondrial DNA (mtDNA) sequences from 995 Chileans representing populations living at different longitudes. The data indicate that the majority of the mtDNAs are of Native American origin (88%). The remaining haplotypes are mostly of recent European origin (11%), and only a minor amount is of recent African ancestry (1%). While these proportions of continental ancestries are very homogeneous along the country, the differences show-up when examining the variation in more detail. Thus haplogroup A2 reaches >10% in the North, and its frequency decreases gradually to 1% in the southern cone; while the frequency of haplogroups D1/D4 follow the opposite pattern. Moreover, there are clear signatures of founder effects in profiles of Native American and European origin. Different measures of molecular

diversity indicate higher values in the North decreasing southward.

94. ANALYSIS OF Y CHROMOSOME HAPLOTYPES IN CHILE: A COMPREHENSIVE NATIONAL STUDY

Toscanini U.[1,2], Moreno F.[2,3], Pantoja-Astudillo J. A.[3], Aguirre Morales E.[3], Bustos P.[3], Pardo-Seco J.[2], Salas A.[2]

[1] *Pricai-Fundación Favaloro, Buenos Aires, Argentina*
[2] *Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain*
[3] *Servicio Medico Legal, Ministerio de Justicia, Santiago, Chile*
[4] *Infectious Diseases and Vaccines Unit, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain*

antonio.salas@usc.es

In the context of the Human Rights Program of the Medico Legal Services of Chile, we analyzed the Y chromosome haplotypes of 978 non-related male individuals grouped by five sampling regions representing the main geo-political regions of the country. Here we report the main findings related to the haplotype distribution for 17 Y-STRs (Yfiler) in Chile. Overall, 803 different haplotypes and 688 singletons were observed. Haplotype diversity was above 0.999 except for the southern cone region, which also exhibited the lowest Discrimination Capacity (0.809) and the highest Matching Probability (0.0084). These results are in good agreement with mtDNA data, suggesting that there is a gradual lost of diversity along the North-South longitudinal geographical axis. As expected, all the parameters computed showed an increased capacity of the Yfiler haplotypes when compared with the results for the minimal haplotype in the same individuals. Beyond the main goal of this project, comparisons among these samples were performed as well as with samples from other American and worldwide locations in order to have a more detailed knowledge of the male background origin of the Chileans. These comparisons revealed that among variation within Chile accounts for 0.25%, and molecular diversity is lower than in other neighboring countries (e.g. Argentina). R_{ST} distances show an important proximity of Y-chromosome profiles to European ones. When compared to mtDNA profiles, this reveals a very strong gender-bias, more extensive than the one reported in other American countries.

95. THE EXTINCT MITOCHONDRIAL DNA CLADE RETRIEVED FROM A 500-YEARS-OLD SOUTH AMERICAN INCA MUMMY

Gómez-Carballa A.[1,2], Catelli L.[4], Martinón-Torres F.[2,3], Vullo C.[4], Salas A.[1,2]

[1] *Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain*
[2] *Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain*
[3] *Infectious Diseases and Vaccines Unit, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain*
[4] *Equipo Argentino de Antropología Forense, Independencia 644 - 3A, Edif. EME1, Córdoba, Argentina.*

antonio.salas@usc.es

In 1985, a frozen mummy was found in Cerro Aconcagua (Argentina). Diverse archaeological studies identified the mummy as a seven-years-old Inca sacrifice who lived >530 years ago, at the time of the expansion of the Inca Empire towards the southern cone. Its mitochondrial DNA (mtDNA) control region haplotype was obtained by two independent laboratories. The results were cross-compared and matched completely. The DNA of the mummy was well preserved as indirectly indicated by the good quality of the sequencing results obtained in both laboratories. The entire mtDNA genome could be completed. By querying a large database of mitogenomes (>28.000) we could not find closely related phylogenetic neighbours. The profile of the mummy represents a still unreported phylogenetic branch within haplogroup C1b, namely C1b15*i*, different to the many that have been already described

in the literature. The expansion of C1b in the Americas dates to the initial human continental settlements (about 18.000 y.a.); however, its internal sub-haplogroup variation differs in Mesoamerica and South America. By querying large databases of control region haplotypes (>150.000), we could only find closely related matches: one haplotype obtained from ancient DNA of an individual belonging to the Wari Empire (Peruvian Andes), and four haplotypes sampled in Bolivia (three of them from Andean Aymara). Overall, the results suggest that the profile of the mummy represents an extinct (or very rare sub-clade nowadays) that could be more frequent in the Andes at that time. A Peruvian Inca origin of C1b15*i* would satisfy both the genetic and paleo-anthropological findings.

96. DETECTING THE PATERNAL GENETIC DIVERSITY IN WEST AFRICA USING Y-STRS AND Y-SNPS

Oliveira A. M.[1], Gusmão L.[2,3,4], Schneider P. M.[5], Gomes I.[5]

[1] *Genetic Department, Institute of Biology, State University of Rio de Janeiro (UERJ), Brazil*
[2] *DNA Diagnostic Laboratory (LDD), Institute of Biology, State University of Rio de Janeiro (UERJ), Brazil*
[3] *IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto (Porto), Portugal*
[4] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto (Porto), Portugal*
[5] *Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany*

lgusmao@ipatimup.pt mailto:

This study aimed to characterize the paternal genetic background in the West African region using 17 Y chromosomal STR loci and 31 Y chromosomal SNPs. A high Y-STR haplotype diversity was observed (1.0000±0.0018; n=86), which was similar to that obtained for samples from other African countries. Haplotype frequencies were compared between populations from West Africa and 13 other African countries. The results revealed no significant differences with 10 of the populations studied, except with population samples from Uganda, Kenia and South Africa. In the analysis of the MDS plot, a cluster was observed with high proximity between our sample from West Africa and other countries from the same region harboring a high proportion of Bantu groups (Nigeria, Benin and Ivory Coast). The majority of the Y-SNP lineages found in our sample set belong to haplogroup E (M2-derived allele) that is typically observed in sub-Saharan Bantu populations (55.8%), followed by haplogroup E with M191 derived allele (32.56%) and just two chromosomes belonging to haplogroups that are rare in Africa (the typically European groups R1b1-P25 and I M70). The haplogroup diversity was 0.6895±0.0200, which is at the same level of previously described diversities in other Bantu populations. In conclusion, a high diversity of paternal lineages was found in Western Africa, showing no significant differences with populations from other regions in Africa, mainly represented by Bantu groups. In contrast, significant differences were found with samples from Eastern and Southern Africa that present an ancestry influence mostly by Nilo/Saharan and Khoisan groups.

97. MOSAIC MATERNAL ANCESTRY IN THE GREAT LAKES REGION OF EAST AFRICA

Gomes V.[1], Pala M.[2], Salas A.[3], Álvarez-Iglesias V.[3], Amorim A.[1,4], Gómez-Carballa A.[3], Carracedo Á.[3], Clarke D. J.[2], Hill C.[2], Mormina M.[5,6], Shaw M. A.[5,7], David D.[8], Pereira R.[1], Pereira V.[9], Prata M. J.[1,4], Sánchez-Diz P.[3], Rito T.[10], Soares P.[11], Gusmão L.[1,12], Richards M. B.[2,5]

[1] *Institute of Molecular Pathology and Immunology of University of Porto (IPATIMUP), Porto, Portugal*
[2] *Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, UK*
[3] *Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain*[4] *Faculty of Sciences, University of Porto, Portugal*
[5] *Faculty of Biological Sciences, University of Leeds, UK*
[6] *Department of Applied Social Studies, University of Winchester, UK*
[7] *Leeds Institute of Molecular Medicine, Faculty of Medicine*

and Health, University of Leeds, UK
[8] Department of Pathology, University of Cambridge, UK
[9] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[10] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal
[11] Centre of Molecular and Environmental Biology, University of Minho, Braga, Portugal
[12] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil

m.b.richards@hud.ac.uk; lgusmao@ipatimup.pt; antonio.salas@usc.es

The Great Lakes lie within a region of East Africa with very high genetic diversity, home to many different ethno-linguistic population groups, who are usually assumed to be the product of a small number of major dispersals of Bantu and Nilotic speakers. Our knowledge of these dispersals relies primarily on the inferences of historical linguistics and oral traditions. Here, we compare mitochondrial DNA (mtDNA) lineages at this putative genetic crossroads between 409 representatives of the major language groupings: Bantu speakers and Eastern and Western Nilotic speakers. We show that Uganda harbours some of the highest mtDNA diversity in Africa, situated on the cusp of East and Central Africa, and also that, despite strong evidence for gene flow between them, the various linguistic groups are significantly differentiated from each other. In spite of an inferred origin for the languages in southern Sudan, the two Nilotic-speaking groups show no sign of a common source mtDNA pool, with most sharing between them evidently due to recent gene flow. The Eastern Nilotic group largely carries lineages characteristic of other Eastern African populations, albeit with a significant signal characteristic of Central Africa, primarily due to Bantu influence, whereas the Western Nilotic group more closely resembles Central African populations. The Bantu speakers share lineages with both Nilotic groups, albeit more similar to Western than Eastern Nilotic speakers, but they also share Eastern African lineages not found in Western Nilotic speakers, likely as a result of assimilating indigenous populations since arriving in the region ~3000 years ago.

98. ASSESSING THE GENETIC DIVERSITY IN THE EXTANT CHACHAPOYA POPULATION FROM NORTHEASTERN PERU USING UNIPARENTAL DNA MARKERS

Guevara E.[1], Palo J.[1], Sajantila A.[1], Guillén S.[2]

[1] Department of Forensic Medicine, University of Helsinki, Finland
[2] Centro Mallqui, Institute of Bioarchaeology, Peru

evelyn.guevara@helsinki.fi

Our aim is to elucidate the origin and population history of the human communities that inhabit the territory of the ancient Chachapoya (800-1470 C.E.) in northeastern Peru, with both contemporary and ancient DNA data. Still in the 16th century this area hosted a rich Chachapoya culture that 'vanished' in the late 17th century, the origin of which remains a mystery. Here we report results and conclusions from the study of the contemporary genetic diversity.

We used 23 Y-chromosomal STRs and mtDNA sequence (HVR1 and HVR2) data from four Peruvian populations: Chachapoya, Huancas, Jivaro and Cajamarca. The main target population, the Chachapoya, has high levels of diversity e.g. Y-chromosome data (h=0.9974±0.0032) and interestingly shows signatures of population expansion for both markers e.g. unimodal mismatch distribution pattern, large Tajima's D (D=-1.51132, p=0.0309) and Fu's Fs (Fs=-23.98616, p=0.0018) values. For mtDNA data, the Chachapoya has short genetic distances ($F_{ST} \leq 0.05$, $p \leq 0.05$) with several Peruvian (e.g. Ancash, Yungay) and other Native Americans (e.g. Embera, Coya). With respect to mtDNA variation, the Chachapoya does not cluster with Andean nor Amazonian groups but rather assumes a basal position among most South American populations. For Y-chromosome, the Chachapoya exhibits short genetic distances only with a Peruvian sample (F_{ST} =0.052, p<0.0001) and with the Mapuche from Chile (F_{ST}=0.049, p<0.0001). In South America, few populations from the Andean-Amazonian divide have been genetically characterized to date. Our results point to a complex and distinctive past demographic history in the Chachapoya

from northeast Peru, one of such populations.

99. OF ALPS AND MEN: ON SOCIAL AND Y CHROMOSOMSOMAL MARKERS FROM TYROL, AUSTRIA

Niederstätter H.[1], Berger B.[1], Erhart D.[1], Parson W.[1,2]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[2] *Forensic Science Program, The Pennsylvania State University, PA, USA*

harald.niederstaetter@i-med.ac.at

Alpine landscapes impede human dispersal and both migration and permanently habitable areas are largely restricted to mountain valleys. The complex topography results in complex patterns of human settlement characterized by communities with differing degrees of size and connection, even within regions of limited range. This, very likely, will leave its mark on the local genetic make-up, as successful movement of individuals between communities forms a basis of genetic diversity. On top of that, social factors, such as inheritance law fostering patrilocality or authoritarian restrictions in access to marriage and reproduction, can form barriers with low permeability to gene flow and will support non-random mating. In general, larger and/or more connected communities will be less susceptible to the interplay of these different factors, whereas small and isolated groups are at a higher risk of becoming subject to the effects of genetic drift and losing genetic diversity.

On this background, we set out to characterize differences and commonalities between both small, in historical terms rather isolated and large more connected municipalities from all parts of Tyrol. Personal information as reported by the voluntary study participants on their paternal grandfathers was utilized to assess familial migration history and used to estimate the degree of patrilocality. Y-chromosomal SNPs and STRs provided a molecular genetic perspective on male population composition and surnames served as cultural markers of ancestry.

100. ASYMMETRIES IN MATERNAL AND PATERNAL GENETIC CONTRIBUTION IN MENDOZA POPULATION AND THE ROLE OF AFRICANS

Marino M.[1,2], Locarno L.[1,2], Furfuro S.[1]

[1] *Laboratorio de Análisis de ADN, School of Medical Sciences, National University of Cuyo, Mendoza, Argentina*
[2] *Laboratorio de Genética Forense, Poder Judicial de Mendoza, Mendoza, Argentina*

mmarino@fcm.uncu.edu.ar

The population of Mendoza was historically considered a heterogeneous population with a predominantly Caucasian population. In recent years, several scientific studies have demonstrated that the population of Argentina has also an important Amerindian contribution. On the other hand, in Mendoza we must add the African contribution. Africans were brought to work as slaves and to participate actively in most military campaigns.

In order to know the ethnic composition of Mendoza's population, 230 unrelated male individuals of Mendoza were analyzed using uniparental Ancestry Indicative Markers (AIMs)

We analyzed 17 Y-STRs, Y-SNPs (M269, M304, P257, U179, M242, M3, M145) and the complete sequencing of mitochondrial D-Loop.

The Y-chromosome results suggest that the majority of the population of Mendoza belongs to one of the major European Caucasian haplogroups (76%), 9% to Amerindian ancestry and surprisingly 10% to African haplogroups.

The maternally inherited haplogroups show a high percentage of Amerindian ancestry (65 %), and a minority of Caucasian (21%) and African (5%) ancestry.

The considerable proportion of African contribution, higher than in other regions of Argentina, is due to the large number of black slaves and the arrival from Africa of male to be used as soldiers in the "Ejercito de los Andes" (The Andes Army) led by General San Martín, during the early XIX century. In addition, after the "freedom of wombs" (1813) and the abolition of slavery

(1853) in Argentina, Mendoza became the crossing point for slave trade to Chile, during the gold rush in California.

101. EXPLORING REGIONAL PATTERNS OF Y-CHROMOSOME VARIATION IN ARGENTINA

Toscanini U.[1,3], Vullo C.[2], Berardi G.[1], Llull C.[1], Borosky A.[2], Gómez A.[1], Pardo-Seco J.[3], Salas A.[3]

[1] *PRICAI-Fundación Favaloro, Buenos Aires, Argentina*
[2] *LIDMO-EAAF, Córdoba, Argentina*
[3] *Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain,*

utoscanini@pricai.com.ar; antonio.salas@usc.es

A study of 23 Y-STRs (Powerplex®Y23 system) was conducted in 257 individuals living in urban areas of eight Argentinean provinces. The data have been meta-analyzed with 364 profiles obtained from the YHRD that represent other five provinces from the country. Standard diversity indices and parameters of forensic interest were computed and population comparisons among the studied populations (*AMOVA* and R_{ST}) were performed. A total of 255 different haplotypes were observed (253 singletons). Duplications, null alleles and several intermediate alleles were detected at different markers. Overall, diversity indices were slightly higher than those reported for other Argentinean populations. Genetic structure as estimated from *AMOVA* and considering all the Argentinean populations available and exploring different grouping scenarios, yielded high within population variance. Moreover, *AMOVA* results were comparable when obtained from the 23-Y-STR-haplotypes versus the minimal haplotypes. Not surprisingly, analyses of genetic distances (R_{ST}) with respect to main ancestral continental populations indicate the Argentinean profiles to be closely related to those from Europeans. The results from this study provide a quite complete picture of the Y chromosome variation in Argentina, notably contributing to increase previous datasets, and therefore allowing a better estimation of parameters of interest in forensic casework and parentage testing.

102. MITOCHONDRIAL DNA: 30 SNPS ARE ENOUGH TO DISCRIMINATE AN ADMIXED POPULATION FROM ESPIRITO SANTO STATE, BRAZIL

Ambrosio I. B.[1,2], Braganholi D. F.[1], Polverari F. S.[1,2], Cicarelli R. M. B.[2]

[1] *Instituto de Química, Universidade Estadual Paulista – UNESP, Araraquara, São Paulo, Brazil*
[2] *Laboratório de Investigação de Paternidade, FCF-NAC, Universidade Estadual Paulista – UNESP, Araraquara, São Paulo, Brazil*

cicarell@fcfar.unesp.br

Mitochondrial DNA (mtDNA) analysis has proved to be useful for forensic identification, especially in cases which nuclear DNA markers fail, as in degraded samples or in cases where the biological material has few traces or no nuclear DNA. Moreover, it can be applied in population genetics, inferring the origin of a such population. In this work, thirty (30) single nucleotide polymorphisms (SNPs) of mtDNA were analyzed in 100 samples of unrelated individuals born and living in the Espirito Santo state, Brazil, which allowed the classification in haplogroups. The results showed 19 haplogroups in this population classified according to its origin: 43% African, 30% European, 26% Native American and 1% Asian. L3 was found the main haplogroup. The results were very similar to previous work in our laboratory, using the entire mtDNA control region (1) which analyzed 97 individuals: African (43.3 %), European (32.0 %), Native American (23.7 %) and Asian (1.0 %). Haplogroup distribution analysis confirmed a highly admixed Latin American population. This type of tool can be used to study different human populations, such as highly admixed as well as Brazilian population, and also in the study of migration's history and colonization of different states and countries of the world.

Acknowledgments: PADC (2013/6-I); FAPESP (2013/12282-0); PROPE; PROPG.

1. Sanches N.M., Paneto G.G, Figueiredo R.F., Mello A.O., Cicarelli R.M.B.: Mitochondrial DNA control region diversity in a population from Espirito Santo state, Brazil. Molecular Biology Reports. 2014; Volume: 41 page 6645 – page 6648.

103. POPULATION GENETIC DATA AND CONCORDANCE STUDY FOR THE POWERPLEX Y23 SYSTEM AND THE AMPFℓSTR YFILER KIT IN KOREANS

Hwang J. H.[1], Cho N. S.[2], Nam Y. H.[3], Kang P. W.[3], Park K.[3]

[1] Daegu institute, National Forensic Service, Waegwan, Korea, Republic of
[2] Seoul institute, National Forensic Service, Seoul, Korea, Republic of
[3] National Forensic Service, Wonju, Korea, Republic of

cleanpond@korea.kr

Haplotypes and allele frequencies of 23 Y-chromosomal STR loci were included in the PowerPlex® Y23 system and the AmpFℓSTR® Yfiler™kit were obtained from a sample of 301 unrelated individuals living in South Korea. The PowerPlex® Y23 system configuration contains 17 loci of the AmpFℓSTR® Yfiler™ kit and 6 new recommended loci (DYS576, DYS481, DYS549, DYS533, DYS570, and DYS643). A total of 300 haplotypes were observed in the 301 individuals studied, of which 299 were unique. The overall haplotype diversity for the 23 Y-STR loci was 0.99998, and the discrimination capacity was 0.99668. We found 10 atypical alleles, including null, duplicated, triplicated and microvariant alleles. Especially, DYS570 19.1 allele and 19.3 (5 individuals, 1.7%) are frequently observed in our study. The concordance study made for this population data revealed no discordant allele between the kits. The results indicate that these 23 loci are useful genetic markers for forensic personal identification and paternity testing in the Korean population.

1. M. Nei: Molecular Evolutionary Genetics. New York: Columbia University Press; 1987: 176-179
2. L. Excoffier, G. Laval, S. Schneider: Arlequin ver 3.0: an integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1; 2005: 47-50
3. J.H. Hwang, H. Lee, Y.A. Lee, M.S. Han, W. Kim, I. Jin, N.S. Cho: Haplotypes for 12 Y-chromosomal STR loci in a Korean population(the central region). Forensic Sci. Int. 2007; 168: 73-84.

104. GENOTYPING OF 52 Y-CHROMOSOMAL HAPLOGROUP SPECIFIC SNPS FOR SKELETAL REMAINS FROM AN EXCAVATION FIND FROM THE MIGRATION PERIOD

Pflugbeil A. M.[1,2], Harthun M.[1], Bruchhaus H.[1], Edelmann J.[1], Labudde D.[2], Dreßler J.[1], Thiele K.[1]

[1] Institute of Legal Medicine, Johannisallee DE-04103 Leipzig
[2] University of Applied Sciences – Forensic Science Investigation Lab (FoSIL), Technikumplatz 17, DE-09648 Mittweida

Anne-Marie.Pflugbeil@medizin.uni-leipzig.de

Analyses of the genetic heterogeneity between populations are often based on common Unique Event Polymorphism (UEP), like Single Nucleotide Polymorphisms (SNPs). On the male scale, changes in paternal lineages where only represent by specific mutational events in the Y-chromosome which are able to reflect ancient population structures and migration events and provide interesting news about historical excavation finds. We analyzed the Y-chromosome of 16 males of an excavation find from the Migration Period (1.500 AD). The skeletal remains were found in the near of a little village in Saxony-Anhalt. Ancient DNA (aDNA) was isolated and purified from prepared tooth samples from each individual. For Y-chromosomal SNP typing, a set of 52 haplogroup YSNPs, that are specific to the most of the main haplogroups of the ISOGG phylogenetic tree from 2014, were analyzed. Overall we classified

42 Y-chromosomal haplogroups by the application of the mentioned multiplex system. The most represented Y-chromosomal haplogroups in the analyzed population were central Europe specific macro haplogroups I (31 %) and R (32 %). In Order, 12 % of the samples were classified in halogroup K. 6 % of the male samples could be classified into super haplogroup CF. 19 % of the remaining male samples were typed into super haplogroup BT. To draw conclusions about time depended changes in haplogroup distributions, we compared the resulted haplogroup distribution with modern hg frequencies of different populations.

105. Y-SNPS GENETIC VARIATION IN THE CAUCASIAN POPULATION LIVING IN SOUTH PORTUGAL

Gaudêncio S.[1,2], Costa H. A.[1,4], Amorim A.[1,3,4], Ribeiro T.[1,4], Porto M. J.[1,4], Santos J. C.[1,2,4], da Silva C. V.[1,4]

[1] *Instituto Nacional de Medicina Legal e Ciências Forenses, Serviço de Genética e Biologia Forenses, Portugal*
[2] *Faculdade de Medicina, Universidade de Lisboa, Portugal*
[3] *Faculdade de Ciências, Universidade de Lisboa, Portugal*
[4] *CENCIFOR – Centro de Ciências Forenses, Coimbra, Portugal*

The Y chromosome has unique characteristics, namely the genetic non-recombination and the direct father/ son transmission. Binary polymorphisms located on the non recombining portion of the human Y chromosome (Y-SNPs) have been widely used for characterizing and classifying paternal lineages in various fields such as genealogy, anthropology and forensics. In anthropological context, Y-SNPs have importance for ancestry studies, allowing the study of migration phenomena and also human evolution history reconstruction.

The population genetic studies can be conducted using Y-SNPs (single nucleotide polymorphisms), biallelic markers which consist of a single gene mutation in a given genomic position of the Y chromosome. These genetic markers contribute to obtaining information related to migration patterns and human biogeographical ancestry.

The application of these markers in biogeographical studies, implies the characterization of haplogroups with different markers, according to geographic region under study. In this research was used a multiplex with the following markers: 92R7, M70, M22, Tat, P25, SRY1532, M173, M213, M9.

A sample of 100 unrelated caucasian individuals, living in south Portugal, was selected for this study. Bloodstains were extracted by Chelex® method [1]. The Y haplogroup PCR reaction was performed with QIAGEN® Multiplex PCR kit using an Veriti® 96-Well Thermal Cycler amplification system. The amplified products were purified with Exo-SAP-it® (USB®), followed by minisequencing reaction performed with the SNaPshot Multiplex kit ™ Kit (Lifetech) according to manufacturers protocol [2]. Sequenced products were purified with SAP (USB®), analyzed in an automatic sequencer ABIPrism®3130 Genetic Analyzer and analyzed with GeneMapper 3.2.1 software. (Applied Biosystems). Haplogroup frequencies were calculated with SPSS. Our preliminary results revealed that the most frequent haplogroup in Caucasian males from south Portuguese Caucasian population is haplogroup R1.

106. ANALYSIS OF Y CHROMOSOMAL STRS IN SOUTHERN OF PORTUGAL POPULATION WITH POWERPLEX Y23

Cruz M.[1,2], Ribeiro T.[1,3], Porto M. J.[1,3], Costa Santos J.[1,2,3], Carvalho M.[1,3]

[1] *Forensics Genetic Service, Southern Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Lisboa, Portugal*
[2] *Faculty of Medicine, University of Lisboa, Portugal*
[3] *CENCIFOR – Forensic Sciences Centre, Portugal*

mariajoao.porto@dcinml.mj.pt

Short tandem repeats (STRs) became a vital tool in forensic genetics due to its high level of polymorphism and abundance in the human genome.

Y-chromosome short tandem repeats (Y-STRs) specifically, play an important role in sexual assault cases, where the female component is in greater proportion than the male component, in complex paternity testing of male children and in cases of missing persons or disaster victim identification, involving profiles of male relatives. The PowerPlex®Y23 System is a five-dye Y-STR multiplex that allows simultaneous genotyping of 23 Y-STR loci (DYS576, DYS389I, DYS448, DYS389II, DYS19, DYS391, DYS481, DYS549, DYS533, DYS438, DYS437, DYS570, DYS635, DYS390, DYS439, DYS392, DYS643, DYS393, DYS458, DYS385a/b, DYS456 and Y-GATA-H4). In this study, samples of 243 unrelated male individuals living in the southern of Portugal were amplified, according to the manufacturer's recommendations, in a GeneAmp® 9700 Thermal Cycler. PCR products were separated and detected on an ABI PRISM® 3130 xl and the electrophoresis results analyzed with GeneMapper® ID software v1.2. Allele frequencies and haplotype frequencies were estimated and compared with frequencies from other European population.

107. STUDY OF Y-SNPS GENETIC MARKERS WITH FORENSIC INTEREST AND ANCESTRY INFORMATIVE POWER IN PALOP'S IMMIGRANT POPULATIONS IN LISBOA - PRELIMINARY RESULTS

Inácio A.[1,2], Afonso Costa H.[1,6], Vieira-Silva C.[1,6], Ribeiro T.[1,6], Porto M. J.[1,6], Costa Santos J.[1,3,4,6], Igrejas G.[2], Amorim A.[1,4,5,6]

[1] *Serviço de Genética e Biologia Forenses, Delegação do Sul, Instituto Nacional de Medicina Legal e Ciências Forenses, Lisboa, Portugal*
[2] *Departamento de Genética e Biotecnologia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
[3] *Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal*
[4] *Instituto Superior de Ciências da Saúde Egas Moniz, Monte da Caparica, Portugal*
[5] *Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal*
[6] *CENCIFOR - Centro de Ciências Forenses, Coimbra, Portugal*

mariajoao.porto@dcinml.mj.pt

The increasing number of immigrants in Portugal is an unavoidable reality. According to Portugal Contemporary Base - PORDATA -, by the end of 2013, the total number of immigrants from PALOP (Portuguese-speaking African countries) in Portugal was about 100 000, and from those, about 75 000 are part of Lisboa population. The migratory phenomenon in Portugal can became one of the main factors for the genetic variability.

Markers located on the Y chromosome have special interest and application in origin and evolution population studies, because most of the chromosome does not undergo recombination. Y-SNPs are single nucleotide polymorphisms with ancestry, population and forensic applications.

Since there is no data for Y-SNPs markers of PALOP immigrants living in Lisboa, our aim is the characterization of those groups of individuals by typing them with a panel of Y-SNPs proposed by Rosser and collaborators in 2000. A set of 9 Y-SNPs markers was typed in order to compare different groups of individuals/populations. Thus, we study 200 bloodstain samples belonging to immigrant individuals from Angola, Guinea-Bissau and Mozambique.

DNA extraction was performed with Chelex® 100 and amplified in a multiplex PCR with primers for 9 Y-SNPs.

Through the results obtained we can confirm that African populations exhibit some differences between them and the Portuguese population. However when we compare the results within each African population only a few differences are shown with the markers used in the present study, which reveals that this Y-SNPs panel is not useful for differentiation purposes within African populations.

108. GENETIC LINK BETWEEN CHAOSHANESE AND OTHER CHINESE POPULATIONS: EVIDENCE FROM MITOCHONDRIAL DNA

Xu K. K.[1], Yao Y. G.[2], Hu S. P.[1]

[1] *Molecular Biology and Forensic Genetics Laboratory,*

Shantou University Medical College, Guangdong, China
[2] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan, China.

sphu@stu.edu.cn

To clarify the matrilineal genetic composition of Chaoshan population and its relationship with other Chinese populations, we investigated the mitochondrial DNA of 256 Chaoshan individuals. The mtDNA HVS-I sequence and haplogroup frequency data of other Chinese populations were collected and used for population comparison. Population relationships were examined by principal component, neighbor-joining phylogenetic tree and median-joining network analyses. In addition, admixture analysis was performed to estimate the relative contribution of the northern Han and southern natives to the Chaoshan population. Our results indicated that the Chaoshan population, along with other southern Han, was well separated from the northern Han and occupied an intermediate position between northern Han and southern natives. In the matrilineal gene pool of the Chaoshan population, genetic composition of southern natives and northern Han took up about 50% respectively. Our results support both northern Han and southern natives origin of Chaoshanese and additionally reveal that during the northern Han migration southward, social factors such as patrilocality, mate choice, cultural assimilation and sex ratio of migration, might have played significant roles in shaping the matrilineal genetic landscape of the Chaoshanese.

109. FOUNDING MOTHERS OF CHUETA POPULATION

Ferragut J. F.[1], Marques S. L.[2], Ramon C.[1], Castro J. A.[1], Amorim A.[2,3], Alvarez L.[2,3], Picornell A.[1]

[1] Institut Universitari d'Investigació en Ciències de la Salut (IUNICS) i Laboratori de Genètica, Departament de Biologia, Universitat de les Illes Balears, Palma de Mallorca, Illes Balears, Spain
[2] Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), Porto, Portugal
[3] Faculty of Sciences of the University of Porto, Porto, Portugal

jf.ferragut@uib.es

Chuetas are the descendants of the condemned crypto-Jews in the last Inquisition processes in Majorca (Balearic Islands, Spain) in the 17th century. Despite their conversion to Catholicism, they were discriminated against and secluded by their immediate neighbors and, consequently, have had strong endogamic behavior until the middle of the twentieth century [1, 2]. Currently, Chuetas are the only Spanish population whose ancestors can be traced to the original Sephardic Jewish populations. In the present work we analysed 109 non-related Chueta individuals, aiming to investigate their maternal lineages. Two mitochondrial DNA (mtDNA) fragments were amplified and sequenced using mtDNA-specific primers in order to obtain the entire control region (16024-576). Haplogroup classification was performed according to current nomenclature (Phylotree Build 16) [3]. High haplogroup diversity was found in Chuetas, indicating that their inbreeding has not implied an impoverishment of their maternal lines. The Middle-Eastern haplogroup R0a was the most prevalent (19.3%), followed by the widespread European haplogroup H (16.5%). The haplogroups that stood out in the Chueta sample, when compared to the Majorcan host population, were: R0a, T1a, T2, K1a, U1a and L3e, indicating a remarkable signature of Middle Eastern ancestry along with some degree of European and North African admixture. These data confirm that the Chuetas, due to their singular history, have kept not only the memory of their Jewish origin through centuries but also a substantial degree of ancestral genetic signature. These significant differences observed between Chuetas and the host population should therefore be taken into account in forensic casework.

1. Braunstein B: The Chuetas of Majorca. Conversos and the Inquisition of Majorca. New York: Ktav Publishing House, Inc; 1936
2. Moore K: Those of the street. The Catholic Jews of Mallorca. Indiana: University of Notre Dame, 1976

3. van Oven M and Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 2009; 30(2): E386-E394

110. COMPARING DIFFERENT POPULATION GROUPS IN SANTANDER – COLOMBIA THROUGH Y-STR HAPLOTYPE ANALYSIS

Castillo A.[1], Rueda K.[1,2], Pico A.[1], Gil A.[1], Vargas C.[1], Rondón F.[2], Gusmão L.[3,4,5]

[1] Genetics Laboratory, Industrial University of Santander, Bucaramanga, Colombia
[2] Conservation Genetics Laboratory, Industrial University of Santander, Bucaramanga, Colombia
[3] DNA Diagnostic Laboratory (LDD), Institute of Biology, State University of Rio de Janeiro (UERJ), Brazil
[4] IPATIMUP, Institute of Pathology and Molecular Immunology of the University of Porto, Portugal
[5] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

castillo@uis.edu.co

Colombia is a multi-ethnic country subdivided into 32 departments. One of this is Santander, which is located in the North East region of the country and divided into six provinces. With the aim of characterizing the Y chromosome genetic pool of Santander, in this study we collected 352 samples of unrelated males from the six provinces: Soto, Mares, Guanentina, Comunera, García Rovira and Velez. These samples were typed for 12 Y chromosome specific STR loci (DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS439, DYS437 and DYS438) in a single PCR amplification multiplex. Results showed a high diversity in all province samples. A total of 285 different haplotypes were found in the whole sample from Santander, from which 264 were unique and the remaining 21 were shared among 2 to 8 individuals. In the total sample from Santander, haplotype diversity was 0.9979±0.0005, with an average value of gene diversity over loci of 0.634±0.327. To see if the Colombian population presents substructure for this set of markers, genetic distances were calculated between samples from different departments, and among the different provinces of Santander department. No statistically significant differences were detected among Santander provinces. However, significant differences were observed between Santander and populations from other departments in Colombia. Therefore, in forensic databasing and in the evaluation of forensic cases, it is important to take into account the significant differentiation of some neighbor departments, due to the presence of different ethnic groups and different proportions of Native American, European and African ancestries.

111. EXPLORING SEPHARDIC LINEAGES IN SÃO TOMÉ E PRÍNCIPE

Nogueiro I.[1,2,3], Neto C.[1,3], Marques S. L.[1,3], Alves C.[1,3], Amorim A.[1,2,3], Gusmão L.[1,3,4], Alvarez L.[1,3]

[1] Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal
[2] Faculty of Sciences, University of Porto, Porto, 4169-007, Portugal
[3] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
[4] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, 21550-013, Brazil

inogueiro@ipatimup.pt

São Tomé and Príncipe are the two main islands of a small archipelago located in the Gulf of Guinea, in the western equatorial coast of Africa. These islands were probably uninhabited at the time of the Portuguese discovery in 1471, but soon after that, the first settlements took place including a great number of Jews. After the Portuguese decree of expulsion, many new-Christians or *Conversos* fled to São Tomé e Príncipe, since the Inquisition was never established there. Several documents attest the continuous movement of new-Christians to these islands, which worked as a refuge from inquisitional prosecutions. With the flourishing of the intercontinental commercial trade mainly led by New Christians, São Tomé became an important stopover for

those who fled from Iberia to South-America. To elucidate the genetic impact of the historical Jewish migrations to São Tomé, we selected a sample of unrelated individuals based on the following criteria: (a) sharing surnames with those included in the historical reports as Jewish migrants (b) showing cultural practices putatively related to the Jewish religion. Both maternal and paternal lineages were investigated using the complete mtDNA Control Region, along with 22 SNPs and 23 STR markers of the male specific region of the Y-chromosome. Moreover, 84 autosomal insertion/deletion markers were analyzed. No population substructure was detected between the alleged Jewish descendants and the host population, showing that no particular database is required in forensic cases in relation to Jewish ancestry, contrasting with previous findings on population heterogeneity when other ethnic criteria were considered.

112. Y-STR HAPLOTYPE BACKGROUND OF PHILIPPINES: COMPARISON WITH OTHER SOUTHEAST ASIAN POPULATIONS

Miranda-Barros F.[1], Gorostiza A.[2,3], Carvalho E. F.[1], González-Martín A.[2], Gusmão L.[1,4,5]

[1] *DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Brazil*
[2] *Departamento de Zoología y Antropología Física, Facultad de Ciencias Biológicas, Universidad Complutense, Madrid, Spain*
[3] *Laboratorio Identificación Genética, GENOMICA S.A.U., Grupo Zeltia, Madrid, Spain*
[4] *Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Portugal*
[5] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal*

lgusmao@ipatimup.pt

Philippines is an archipelago in the Western Pacific Ocean. It is one of the most populated country in Southeast Asia, harboring multiple ethnic groups and cultures. Most of the non-indigenous population is composed by Chinese and Spanish immigrants. In order to characterize the Y-STR composition of Philippines, in the present study we have collected 74 samples from unrelated males from the three main geographical divisions: Luzon, Visayas and Mindanao. These samples were typed for the 27 Y-STR loci included the YFiler Plus kit. A high Y-STR haplotype diversity was found (1.0000±0.0022) with all haplotypes being unique in our sample. Low diversities were found for DYS391 and DYS438 (below 34%) and the multi-loci markers DYS385 and DYF387S1 were among the most diverse ones (above 95%). Since no data is available for the full set of YFiler Plus markers, a smaller set (those included in the Powerplex Y23) was used to calculate genetic distances between Philippines and other Southeast Asian populations. The results showed no significant differences between our samples and two other samples from Philippines, one from the general population (n=161) and the other restricted to Luzon region (n=629) ($F_{ST} \leq 0.0024$; $p \geq 0.1009$). Significant differences were observed in comparisons when testing any of the three Philippines samples to Vietnam (n=45) and Singapore (n=303) populations, the only Southeast populations with available data for the studied markers.

113. ENRICHING THE KNOWLEDGE ON EAST ASIA POPULATIONS: CHARACTERIZATION OF MALE LINEAGES FROM MACAU AND SHANGHAI

Alexandre J. G.[1,2], Huang Q.[3], Marques S. L.[2], Lopez-Parra A. M.[3], Arroyo-Pardo E.[3], Amorim A.[1,2], Alvarez L.[2], Prata M. J.[1,2]

[1] *Department of Biology, Faculty of Science, University of Porto, Portugal*
[2] *IPATIMUP – Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Portugal*
[3] *Dpto. of Toxicology and Legal Medicine, Faculty of Medicine, Complutense University of Madrid, Spain.*

lfernandez@ipatimup.pt

There is still much uncertainty about the demographic history of East Asia human populations, especially from China, where currently 56 ethnic groups are recognized. Within them, the Han is by far the majority group, representing

nearly 92% of the Chinese population. In this work, we have performed the genetic characterization of Y-chromosome diversity in two Han Chinese samples (Shanghai and Macau). Comparisons were then carried on with data from other East Asian populations, to evaluate whether signs could be captured on the different geographical, historical and cultural factors that have shaped the Y-chromosome diversity in a broader geographical context. We have analysed 135 unrelated males, 85 from Shanghai and 50 from Macau. 25 Y-SNPs were examined with two multiplex systems using the SNaPshot™ kit (Applied Biosystems). Samples were also typed for 16 Y-STR markers using the AmpFℓSTR® Yfiler® PCR Amplification Kit (Applied Biosystems). As typically found in East Asia populations, in the two studied samples the large majority of the Y-chromosomes fell into haplogroup O-M175: 89.5% in Macau and 81.5% in Shanghai. High haplogroup and haplotype diversities were detected in both populations, reaching values commonly found in other Han Chinese populations. In China, the lowest diversities were reported in people belonging to some of the many minority ethnic groups, in which the recent history can explain the reduced diversity. Comparative analyses also revealed a remarkable degree of population substructure inside China, but yet the level of genetic differentiation was not found to be correlated with the geographical distance between populations.

114. GENETIC CHARACTERIZATION OF 27 Y-STR LOCI IN THE NATIVE POPULATION OF ASHANINKA FROM PERU

Tineo D. H.[1,2], Loiola S.[3], Paredes F. V.[4], Noli L. R.[1], Amaya Y. C.[4], Carvalho E. F.[3], Gusmão L.[3,5,6]

[1] *Universidad Nacional Mayor de San Marcos; Instituto de Medicina Legal del Perú*
[3] *Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro (UERJ), Brazil*
[4] *Ministerio de Salud del Perú. Centro de Salud Puerto Bermúdez, Oxapamapa, Pasco, Perú*
[5] *Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Portugal*
[6] *Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal*

lgusmao@ipatimup.pt

The South American country of Peru is composed by a highly admixed population, with Native American, European and African genetic contributions. Some Native American groups in Peru underwent low admixture with Europeans or Africans and they have kept much of their culture and their original language. In this work we have studied one of these groups called Ashaninka, for the 25 Y chromosome specific STR markers that were included in the recently released YFiler Plus kit. The samples have been collected from 58 unrelated males belonging to 41 different communities located in the margins of the Amazonian rivers Pichis and Palcazú, in the district of Puerto Bermúdez, Pasco region, Peru. A high Y-STR haplotype diversity was found (1.0000±0.0022) with all haplotypes being unique in the studied sample. Two markers that usually present a high diversity in European populations showed very low values of diversity in the Ashaninka Native Americans, namely the DYS635 (Het=0.2263) and DYS437 (Het=0.1325). On the other hand, the DYS438 showed a much higher diversity in Ashaninka (Het=0.6582) group than that usually found in European populations. Apart from the multi-loci markers DYS385 and DYF387S1, more than one allele was also observed in one sample for DYS518 locus. This study represents the first report of haplotype frequencies for the YFiler Plus markers' set in a Native American population, showing a high diversity of haplogroups and, therefore, demonstrating their usefulness in forensic identification cases.

115. GENETIC POLYMORPHISMS AT 17 Y-STR LOCI IN AN EGYPTIAN POPULATION

Nagai A.[1], Elmadawy M. A.[2], Ishihara T.[1], Yamamoto T.[3], Hara M.[4], Yosef T. A.[2], Gomaa G. M.[2], Hegazy H. M. R.[2], Bunai Y.[1]

[1] *Department of Legal Medicine, Graduate School of Medicine, Gifu University, Gifu, Japan*

[2] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt
[3] Department of Legal Medicine and Bioethics, Graduate School of Medicine, Nagoya University, Nagoya, Japan
[4] Department of Forensic Medicine, Faculty of Medicine, Saitama Medical University, Moroyama, Japan

anagai@gifu-u.ac.jp

Haplotypes and allele frequencies for the 17 Y-chromosomal STR loci, DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448 were determined in a sample of 103 unrelated Egyptian males living in the northern region of Nile delta using the AmpFlSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems). This population was demonstrated 95 haplotypes, of which 90 were unique, one was found in four individuals, one was found in three individuals, and three were found in two individuals. There was one haplotype with no allele detected at the DYS458 locus. The haplotype diversity calculated from the 17 Y-STR loci was 0.9977 and the discrimination capacity was 0.9223. This database of 17 Y-STR loci for the Egyptian population would be useful in forensic examinations and human genetic studies.

116. FORENSIC INTEREST OF THE DISSECTION OF THE MAIN EUROPEAN PATERNAL LINEAGE M269 IN ATLANTIC EUROPE AND IBERIA

Valverde L.[1], Illescas M. J.[1], Villaescusa P.[1], Jimenez S.[2], Jarreta B. M.[3], Zarrabeitia M. T.[4], Olofsson J.[5], Morling N.[5], de Pancorbo M. M.[1]

[1] BIOMICs Research Group, Lascaray Research Center, University of Basque Country UPV/EHU, Vitoria-Gasteiz, Spain
[2] Dpt. Pathology and Chirugie (Legal and Forensic Area), University of Miguel Hernández, Alicante, Spain
[3] Laboratory of Genetics and Genetic Idnetification, University of Zaragoza, Zaragoza, Spain
[4] Forensic and Legal Medicine Area, University of Cantabria, Santander, Spain
[5] Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

marianpancorbo@gmail.com

The current genetic makeup of Europe is the result of many population migrations and settlements influenced principally by climate, cultural progress and the historical conquest of territory.

The genetic evidence provided by the analysis of the Y chromosome, which is a valuable tool for the study of the evolution and distribution of paternal lineages, has revealed that currently a large majority of individuals in Central and Western Europe (40-90%) belong to R-M269 lineage.

We have performed the dissection of S116, the major M269 subhaplogroup in Western and South-Western Europe, in more than 1500 individuals.

The results have provided relevant clues about the controversial evolutionary history of M269 and support the theories proposing the origin of M269 haplogroup in Eastern Europe. However, the subhaplogroup S116 shows frequency peaks and spatial distribution which indicate an origin more westwards. Finally, an outstanding frequency of DF27 sublineage has been found in Iberia, with a restricted distribution pattern inside the north of the Iberian Peninsula and a frequency maximum in the area corresponding to the Franco-Cantabrian refuge, that probably corresponds to the origin of this subhaplogroup.

This fine dissection of the M269 patrilineal lineage potentially increases the forensic utility of Y chromosome haplogroup determination during casework.

117. RESOLUTION OF Y HAPLOTYPES USING THE LIFE TECHNOLOGIES YFILER PLUS VTS PCR AMPLIFICATION KIT

Ender M.[1], Burri H.[2], Borer U.[1], Koller C.[1], Schulthess N.[1], Malik N.[1]

[1] Institute of Legal Medicine, Kantonsspital Aarau, 5000 Aarau, Switzerland
[2] Institute of Legal Medicine, University of Zürich, Switzerland

naseem.malik@ksa.ch

Our group participated in the International study, coordinated by Professor Lutz Roewer in Charité, Berlin, utilizing the Power Plex Y23 kit in 2013. To this end blood samples were obtained, after informed consent, from 639 male blood donors. The selection criteria were that they were of Swiss descent from two generations. The samples were anonymized from the blood donor center and the only information received was the nationalities of the respective father and grandfather.

The samples were typed with the Power Plex Y23 system along with Autosomal STRs using the PowerPlex 21 and NGM SElect PCR Amplification Kits.

From this collective, 78 Persons were typed with concordant Y-Haplotypes in 35 Groups. That is 35 Y-Haplotypes were observed to be shared by more than one person. 30 haplotypes were shared by groups each of two persons, 2 haplotypes were shared by groups of three persons and 3 haplotypes were shared by groups of four individuals.

Each group of two persons and pairwise combinations of the groups with multiple individuals were tested for their relationship status with the Autosomal STRs using the PatCan 2 software. 17 Groups were found to be related as father / son pairs or brothers and 18 groups were found to have Likelihood Ratios of less than one for a relationship of first cousins or nearer.

We will present the results of our analyses of these groups using the Yfiler Plus set of markers and test their efficacy in resolving these Y haplotypes.

Theme 05: Massively Parallel Sequencing

118. COMPARISON OF TECHNIQUES FOR QUANTIFICATION OF NEXT-GENERATION SEQUENCING LIBRARIES

Hussing C., Kampmann M. L., Mogensen H. S., Børsting C., Morling N.

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

christian.hussing@sund.ku.dk

Quantification and dilution of next-generation sequencing (NGS) libraries to equal molar ratios is crucial for optimal efficiency of NGS experiments. Without a well-balanced library pool, some samples may have too few sequences for accurate genotyping or alternatively, it may be necessary to type fewer samples per experiment in order to ensure sufficient coverage for all samples. The different DNA quantification methods vary in accuracy, reproducibility, labour intensity, speed and cost. Eight different methods for DNA quantification were compared to each other by testing quantification of NGS libraries for the Ion Torrent™ and Illumina® platforms. The methods were UV spectrophotometry (Thermo Electron Corporation), Nanodrop (Thermo Scientific), Qubit® (Life Technologies™), Bioanalyzer (Agilent Technologies), Tapestation (Agilent Technologies), GX Touch (PerkinElmer®) and Fragment Analyzer™ (Advanced Analytical). Three qPCR assays were assessed, one based on SYBR®green quantification and two based on probes targeting either the adapter sequence or the amplification products of the SNP locus rs876724 from the SNP*for*ID human identification panel (1). rs876724 is included in the library preparation kits HID-Ion AmpliSeq™ Identity Panel and the ForenSeq DNA Signature Prep Kit for Ion Torrent™ and Illumina® sequencing, respectively. Rather large variations between the methods were observed. The advantages, disadvantages and pitfalls of the various methods will be discussed.

1. Sanchez JJ, Phillips C, Borsting C, Balogh K, Bogus M, Fondevila M, Harrison CD, Musgrave-Brown E, Salas A, Syndercombe-Court D, Schneider PM, Carracedo A and Morling N: A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis. 2006; 27: 1713-1724

119. A CATALOG OF COLLATERAL VARIANTS IN AND AROUND CORE FORENSIC STRS FROM NGS ANALYSIS OF THE HGDP-CEPH DIVERSITY PANEL

Phillips C.[1], Devesse L.[2], Ballard D.[2], Syndercombe Court D.[2], Parson W.[3,4], Carracedo Á.[1,5], Lareu M. V.[1]

[1] Forensic Genetics Unit, Institute of Forensic Sciences, University of Santiago de Compostela, Spain
[2] Department of Forensic and Analytical Science, Faculty of Life Science, King's College, London, UK
[3] Institute of Legal Medicine, Innsbruck Medical University, Austria
[4] Forensic Science Program, The Pennsylvania State University, PA, USA
[5] Galician Foundation of Genomic Medicine (SERGAS), University of Santiago de Compostela

c.phillips@mac.com

Using next generation sequencing (NGS) approaches to genotype forensic STRs brings the opportunity to include SNP and Indel variants present in the repeat region of the STR or between these sequences and the primer binding sites. This additional variation extends the informativeness of the STRs considerably, while allowing differentiation of samples apparently identical-by-state from CE analyses. Such differentiations are particularly useful in mixture analysis, relationship testing and familial searching; where close relatives must be distinguished from coincidental STR allele matches. We genotyped the HGDP-CEPH panel of 944 samples in 52 populations on the Illumina MiSeq FGx™ using the ForenSeq™ DNA Signature Prep Kit, comprising a range of X, Y and autosomal STRs plus autosomal SNPs. Here we report the global patterns of SNP variation found in the 27 autosomal STR sequences generated by the ForenSeq™ Universal Analysis Software; consisting of 23 core loci in widespread use and four NIST mini-STRs. As has already been established from initial NGS studies of smaller sample sets, previously less informative STRs emerge as much more variable forensic markers when collateral variants are included in the data. Furthermore, complex STRs such as D21S11 and D12S391 show many-fold higher discrimination power when their repeat region sequences can be characterised in detail.

120. EVALUATION OF THE RELIABILITY OF THE DATA GENERATED BY TARGETED NEXT GENERATION SEQUENCING FROM ARTIFICIALLY DEGRADED DNA SAMPLES

Carboni I.[1],Fattorini P.[2], Previderè C.[3], Sorçaburu S.[2], Iozzi S.[1], Nutini A. L.[1], Contini E.[1], Pescucci C.[1], Torricelli F.[1], Ricci U.[1]

[1]Diagnostic Genetics Unit, AOU Careggi, Florence, Italy
[2]Dept. of Sciences of Public Health,University of Trieste
[3]Dept. of Public Health, Experimental and Forensic Medicine, University of Pavia

carbonii@aou-careggi.toscana.it

NGS has the potential to be a promising technology for recovering genetic information from challenging specimens in forensic genetics. Nevertheless,the role of DNA damage on the outcome of NGS is practically unknown. For this reason,

we investigated the performances of the Illumina® ForenSeq™ DNA Signature kit (in its pre-commercial version) on a set of in vitro degraded trial DNA samples derived from the aqueous hydrolysis of a single test DNA for 6, 8.5 and 10 hours. After qPCR DNA quantification, duplicate analyses of the degraded samples were carried out by using theDNA Primer Mix A which contained primer pairs for 63 STRs and 95identity informative SNPs. The resultingmolecular products were then sequenced by using the MiSeq® system (Illumina) and analyzed

using ForenSeq Universal Analysis Software (Illumina).The error rate and the coverage of the NGS data obtained from the three degraded samples were compared to the unmodified test DNA.

The NGS data showed that the ability of recovering genotypes and the frequency of analytical artifacts is strongly influenced by the degree of damage of the template.NGS was able to call 17-46% of the STR loci and 26-68% of the SNPs in the trial samples while correct typing was achieved in 4-39% of the STRs and 13-55% of the SNPs.

Our data show that NGS is a powerful method for gathering genetic data from samples which failed the conventional approaches even if the risk of mistyping is not negligible (up to 2%).

121. MASSIVELY PARALLEL SEQUENCING OF GLOBAL STR LOCI IN KOREANS

Kim E. H.[1,2], Lee H. Y.[1], Yang W. I.[1], Shin K. J.[1,2]

[1] Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea
[2] Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Korea

kjshin@yuhs.ac

Because of its capability to produce massively parallel sequencing data and to provide sequence variations, the prospect of NGS in STR typing has been explored by many forensic laboratories using in-house multiplex PCR systems or commercial kits. Meanwhile, the expanded CODIS Core Loci were newly announced to increase the international data compatibility and to maximize the power of forensic DNA databases. Accordingly, we developed a multiplex PCR system for the NGS analysis of 25 forensic markers which include 20 expanded CODIS Core Loci and additional 5 loci (Penta D, Penta E, D6S1043, DYS391 and amelogenin). All amplicons in the multiplex had the size range of 75 to 220 bp, and the barcoded library for the NGS on the MiSeq (Illumina) was easily prepared by 2 rounds of PCR; first round of multiplex PCR using target-specific primers with read sequences, and second round of PCR using primers with indices and platform-specific sequences. To investigate the sequence variations of 24 global STR loci, NGS data of more than 200 Koreans were produced using these simplified workflows. We compared obtained STR genotyping results with those obtained by capillary electrophoresis method, and scrutinized sequence variations in targeted STR region. The observed sequence variations could differentiate same size alleles, thereby increasing the discrimination capability of STR typing. The multiplex PCR system of the present study will facilitate the NGS analysis of global STRs, and the observed sequence variations could be used to store additional information while increasing the discrimination power of STRs.

122. MASSIVELY PARALLEL SEQUENCING OF IDENTIFILER AND POWER PLEX Y AMPLIFIED FORENSIC SAMPLES

England R., Liu A., Curnow N., Stacey J. , Harbison S. A.

Institute of Environmental Science and Research Ltd, ESR Mt Albert Science Centre, Private Bag 92-021, Mt Albert, Auckland, New Zealand

SallyAnn.Harbison@esr.cri.nz

In the last few years the cost of massively parallel sequencing has reduced dramatically to the point that it can now be used as a tool in forensic work. An important consideration for the implementation of any new forensic technology is the ability to remain compatible with previous technology. With this is mind we wanted to know if it was possible to sequence the previously amplified products of two commercial forensic STR multiplexes AmpFlSTR® Identifiler® and PowerPlex® Y using the Illumina© MiSeq and Ion PGM™ Sequencer (Life Technologies) and if so, to begin to characterise the sequence data from a forensic perspective. We found it is possible to sequence such amplified DNA and to accurately determine the STR genotype of forensic samples using both platforms. Sequencing these STR loci provided extra information in the form of sequence variation, something that is not possible measuring amplicon length alone. By characterising features of the DNA sequence profiles, such as

stutter and locus imbalance we identified areas for future development that will be needed before casework implementation. This work was continued with the sequencing of multiple BQA samples and the NIST controls on both the PGM and MiSeq. The results of these comparisons are discussed, including the advantages and limitations of each platform, any bioinformatic problems and solutions and the future directions we are taking.

123. CHEMISTRY AND PERFORMANCE TESTING OF FORENSICALLY-RELEVANT GENETIC MARKER SYSTEMS WITHIN THE ION PGM SYSTEM

Churchill J.[1], King J.[1], Chang J., S[2], Wootten S.[2], Lagace R.[2]
Budowle B.[1,3]

[1] Institute of Applied Genetics, University of North Texas Health Science Center, Ft Worth, TX, USA
[2] Human Identification, Thermo Fisher Scientific, 180 Oyster Point Blvd, South San Francisco, CA, USA
[3] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

bruce.budowle@unthsc.edu

Massively parallel sequencing (MPS) offers substantial improvements over current forensic DNA typing capabilities. One promising approach is the Ion PGM™ System (Thermo Fisher Scientific). This technology relies on integrated semiconductor chip technology for signal detection, has high throughput, and is scalable, all features desirable for the workflow of the application-oriented laboratory. However, for MPS to be implemented, a robust chemistry and forensically-relevant genetic marker systems are necessary. Improvement of the sequencing chemistry through homopolymeric regions may be realized by use of the Hi-Q™ sequencing chemistry, which was evaluated based on performance analysis with whole mitochondrial genome sequence data. Results were compared with whole genome sequence data generated on multiple platforms. Under this new chemistry format a large multiplex short amplicon system that spans the entire mitochondrial genome for analysis of challenged samples was developed. Currently, the mitochondrial panel is comprised of two multiplexes each containing 81 primers pairs and a large number of degenerate primers. The amplicons generated by these primer pairs are ≤175 bps in length. The multiplex efficiency and potential amplicon drop out performance were assessed by comparison with whole genome data form diverse population samples. Metrics described included amplicon balance, coverage, strand balance, amplification success, and sequence concordance. The quality of results supports the utility Ion PGM™ System as a potential platform for forensic applications. This presentation will discuss the strategies for multiplex design with MPS and success and consequences of use of large overlapping short amplicon multiplexes with a focus on forensic applications.

124. AN ASSESSMENT OF THE ILLUMINA MISEQ PLATFORM FOR FORENSIC SNP ANALYSIS

Mehta B.[1,2], Daniel R.[2], Phillips C.[3], McNevin D.[1]

[1] National Centre for Forensic Studies, University of Canberra, Canberra, Australia
[2] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia
[3] Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

bhavik.mehta@hotmail.com

In the last two years, Next Generation Sequencing (NGS) has gained significant forensic interest with the availability of benchtop scale NGS systems. The Illumina MiSeq is an example of such a system and can genotype hundreds of markers from multiple samples in a single run. In this study, a total of 183 identity, ancestry and phenotype SNPs were genotyped for 96 samples. The SNPs were derived from established SNaPshot® (Life Technologies) assays, comprising: the SNP*for*ID 52-plex identity panel [1]; the SNP*for*ID 34-plex [2], Eurasiaplex [3], Pacifiplex (unpublished) and PIMA (unpublished) ancestry panels; and the IrisPlex [4] eye colour phenotyping panel. These six PCR

assays generated the amplified fragments which were then used for library preparation and sequencing. The following forensic criteria were assessed: sensitivity, reproducibility; depth of coverage; throughput; genotyping accuracy; ancestry & phenotype prediction accuracy; effect of environmental insults (UV radiation and humic acid inhibition); effect of DNA extraction methods and the data interpretation process. MiSeq genotypes were >97 % concordant with SNaPshot® genotypes down to 50 pg template DNA. The genotypes produced were highly reproducible and the level of reproducibility was >98 %. Partial genotypes were obtained from UV degraded and HA inhibited samples. The results of these assessments indicate the suitability of the MiSeq system for forensic SNP analysis.

1. Sanchez JJ, Phillips C, Borsting C, et al: A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis. 2006; 27:1713-1724
2. Phillips C, Salas A, Sanchez JJ et al: Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. Forensic Sci. Int. Genet.2007; 1:273-280
3. Phillips C, Freire Aradas A, Kriegel AK, et al: Eurasiaplex: a forensic SNP assay for differentiating European and South Asian ancestries. Forensic Sci. Int. Genet. 2013; 7:359-366
4. Walsh S, Liu F, Ballantyne N, et al. IrisPlex: A sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information. Forensic Sci. Int. Genet. 2011; 5:170-180

126. CONCORDANCE OF MASSIVELY PARALLEL SEQUENCING RESULTS USING THE ILLUMINA MISEQ FGX WITH CURRENT METHODS OF STR TYPING

Devesse L.[1,2], Ballard D.[1], Oldroyd N.[2], Syndercombe Court D.[1]

[1] Faculty of Life Sciences and Medicine, King's College London, 150 Stamford Street, London SE1 9NH

[2] Illumina, Inc., Chesterford Research Park, Little Chesterford, Essex CB10 1XL

Laurence.a.devesse@kcl.ac.uk

Massively parallel sequencing is emerging as the technique of choice to overcome the limitations of capillary electrophoresis (CE) based approaches for DNA typing in forensic science. By using sequencing technology, and simultaneous targeting of autosomal, X and Y STRs as well as identity and phenotypic informative SNPs, the breadth of data obtained from a single run is considerable when considering previous techniques. It is important that this information aligns with STR and SNP data currently obtained using commercially available kits for CE-based investigations, in order for existing databases to be of use to the forensic community. Additional frequency information will also be vital in order to make use of the intra-allelic variability observed through the use of sequencing. We have typed a series of samples using both Promega® PowerPlex ESI 17 and the Illumina ForenSeq™ DNA Signature Prep Kit and MiSeq® FGx in order to compare concordance and population variability. The current work will demonstrate the instances where sequence information can improve the level of discrimination at a targeted marker.

127. EVALUATION OF MASSIVELY PARALLEL SEQUENCING TECHNOLOGIES FOR EXPANDED DNA IDENTIFICATION CAPABILITIES AT THE FEDERAL BUREAU OF INVESTIGATION LABORATORY

Irwin J., Moreno L., Onorato A., Brandhagen M., Callaghan T.

Federal Bureau of Investigation Laboratory, Quantico, VA, USA

Jodi.irwin@ic.fbi.gov

Though Massively Parallel Sequencing (MPS) has transformed numerous genetic disciplines over the past decade, it is only within the past few years that evaluations of MPS for forensic application have been undertaken in earnest.

Given the potential of MPS to not only increase the quantity and discriminatory power of genetic data but also improve the overall throughput of samples through the laboratory, the Federal Bureau of Investigation is exploring the development of MPS assays for possible casework application. Long-term laboratory efforts are directed towards employing MPS as a common platform for testing of all markers of forensic interest. However, near-term efforts are directed specifically towards evaluating the technology for its utility in expanding existing institutional capabilities. Areas of current emphasis are 1) highly challenging samples and the benefits of MPS for improved information recovery 2) mitochondrial DNA typing and the development of entire mitochondrial genome (mtGenome) data in particular and 3) no-subject crime scene samples and the value of ancestry and phenotype markers for developing investigative leads. Commercially available assays designed for forensic application, as well as custom assays for specific nuclear DNA markers and the mtGenome, are currently under evaluation. In addition to its use for these near-term operational applications, MPS is also being used more generally - to better characterize our most challenging samples. With a better understanding of endogenous DNA quantity and quality, forensic examination strategies may be devised that accommodate the challenges of specific case scenarios and therefore broaden the range of sample type and quality from which probative data can be recovered. Here, we present an overview of our general efforts.

128. FORENSIC-LOCI-FINDER (FLFINDER): TOOL FOR ANALYZING FORENSIC LOCI DATA OF SECOND GENERATION SEQUENCING

Zhou N.[1,4], Mao J.[3], Zhu J.[2], Bai X.[3], Wang L.[2], Jiang Y.[2], Liang W.[2], Zhang L.[2]

[1] College of life Sciences, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[3] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China
[4] Bio-resources Key Laboratory of Minister of Education, Sichuan University, Chengdu 610041, Sichuan, China

liangweibo@scu.edu.cn

In past several years, Next Generation Sequencing (NGS) has been used in forensic DNA profile. Different software tools have been developed for analyzing such extensive data. LobSTR and STRait Razor are designed for STR analyzing and MyFlq framework was generated for analyzing STRs and SNPs. Our group has developed microhaplotypes which can only profiled by NGS and has been reported in another abstract. Because of the mentioned tools could not analyze the microhaplotypes, the Perl-based tool Forensic-Loci-finder (FLfinder) was designed to analyzing the microhaplotypes and other forensic loci such as STRs and SNPs. The FLfinder is comprised of FASTQ analyzing part and forensic loci typing part. The first part is used for extracting the sequences which have been sequenced for more than once from the FASTQ data and output the information including sequence and the number of reads. The second part could analyze the sequence obtained from first part. The first part needs to operate on local Windows and Linux whereas it is hard to upload the gigabits data to the Server. The second part could yield the information of STR, SNP, DIP and the Microhaplotypes with the references in the database or submitted by analyzers. The strategy to detect SNP, DIP and Microhaplotypes is naming the sequence first, finding the locus location by references of flank sequence, reading the allele. The strategy to detect STR is naming the locus, reading the length of target region by references of flank sequence, comparing the repeat motif with references.

129. TEST OF THE ILLUMINA FORENSEQ KIT

Hussing C., Børsting C., Mogensen H. S., Morling N.

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Christian.hussing@sund.ku.dk

The Illumnia® ForenSeq™ kit amplifies 27 autosomal STRs, 8 X-STRs, 25 Y-STRs, 95 autosomal human identification SNPs, 56 autosomal AIMs and 24 autosomal SNPs associated with pigmentary traits in one multiplex PCR reaction. The products are sequenced on the MiSeq FGx Forensic Genomics System and analysed with the ForenSeq Universal Analysis Software.

We have tested the sensitivity, reproducibility, phenotypical and ancestry prediction of the assay by typing samples from individuals with known eye, skin and hair colours as well as bio-geographic ancestry. We also present results from typings of challenging samples from crime scenes in order to test the feasability of the assay in forensic genetic crime case work.

130. MISEQ FGX SEQUENCING SYSTEM: A NEW PLATFORM FOR FORENSIC GENETICS

Turrina S., Caratti S., Ferrian M., Cosentino E., De Leo D.

[1] *Department of Public Health and Community Medicine Institute of Legal Medicine, Laboratory of Forensic Genetics, University of Verona, Italy*

stefano.caratti@univr.it

Next Generation Sequencing (NGS) technologies are going to play a major role in forensic genomics in the next few years. A new NGS platform, the MiSeq FGx™ Forensic Genomics System (Illumina Inc., San Diego) was lauched in January 2015 as the first fully validated sequencing system specifically designed for forensic genomics applications. This new system allows simultaneous, high resolution sequencing of 153 identity informative markers (including 27 autosomal STRs, 7 X-chromosomal, 24 Y-chromosomal haplotype markers and 94 SNPs) plus 56 ancestry and 22 phenotypic informative SNPs.

In order to assess the flexibility of MiSeq FGx™ Forensic Genomics System for STR genotyping, a concordance study on samples previously typed using the ABI 3130 Genetic Analyzer with GeneMapper ID-X v1.2 software was performed. Results were compared for 22 autosomal and 27 gonosomal STRs previously analysed and subsequently sequenced using the Forenseq DNA Signature Prep kit and analyzed with the Forenseq Universal Analysis software. Here we present the results of a preliminary study within our laboratory and discuss the likely expectation that the MiSeq FGx™ Sequencing System technology could provide more powerful analytical tools for the forensic genomics field.

131. TEMPLATE PREPARATION OF AMPLISEQ LIBRARIES USING THE ION CHEF

Mogensen H. S.[1], Børsting C.[1], Morling N.[1]

[1] *Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

helle.smidt@sund.ku.dk

The introduction of Next Generation Sequencing (NGS) techniques for forensic use has greatly expanded the possibilities of extracting information from biological stain materials in crime cases. However, the NGS workflows are not fully optimized for continuous high-throughput processing of samples in a forensic genetic laboratory. Although the Ion PGM™ has been on the market since 2010, the process of preparing samples for sequencing is still rather cumbersome and involves a large number of pipetting steps. This creates variability in the loading of the chips and the coverages of the DNA sequences. This variability in turn complicates the interpretation of the results, i.e. low coverage leads to locus or allele dropout for poorly performing markers. In order to automate a part of the sample preparation, we used the Ion Chef™ (Life Technologies) to prepare pooled Ion AmpliSeq™ libraries for emulsion PCR and loading onto sequencing chips.

The workload and the manual number of pipetting steps decreased, while the chip loading efficiency, uniformity of loading and reproducibility increased using the Ion Chef™ compared to the performance obtained with the more manual setup using the Ion OneTouch™ 2 system.

132. ALLELE FREQUENCY AND COVERAGE THRESHOLDS FOR FORENSIC SNP GENOTYPING WITH MASSIVELY PARALLEL SEQUENCING

McNevin D.[1], Mehta B.[1], Phillips C.[2], Daniel R.[3]

[1] National Centre for Forensic Studies, University of Canberra, Australia
[2] Forensic Genetics Unit, University of Santiago de Compostela, Spain
[3] Office of the Chief Forensic Scientist, Victoria Police, Australia

dennis.mcnevin@canberra.edu.au

Massively parallel sequencing (MPS) shows great promise for forensic DNA profiling. We have successfully applied this technology to single nucleotide polymorphism (SNP) genotyping for identity and phenotype prediction1. The development of library barcoding strategies for targeted amplicon sequencing on bench-scale sequencers has resulted in two platforms appropriate for forensic analyses: the Ion PGM™ system (Life Technologies) and the MiSeq FGx Forensic Genomics system (Illumina). To support their platforms, both vendors produce forensic sequencing assays including the HID-Ion AmpliSeq™ Identity and Ancestry Panels (Life Technologies) and the ForenSeq DNA Signature Prep Kit (Illumina). We have previously shown that it is possible to apply customised panels consisting of PCR amplicons from SNaPshot® single base extension multiplex SNP assays (Life Technologies)[1].

There are two important thresholds for properly characterising MPS SNP genotypes. The first is a coverage threshold (number of reads for each amplicon) below which they are ignored for genotyping purposes. This is analogous to the peak amplitude threshold (PAT) employed for capillary electrophoresis. The second is an allele frequency threshold (proportion of reads carrying each allele) below which allelic variants are ignored when calling the genotype. This is analogous to the heterozygous balance threshold employed for capillary electrophoresis. In this study, we investigate the effects of varying both thresholds on the concordance of Ion PGM, MiSeq and SNaPshot SNP genotypes and demonstrate that genotype concordance is relatively insensitive to coverage threshold and to allele frequency threshold in the range 70-90 %.

1. Daniel R, Santos C, Phillips C, Fondevila M, van Oorschot R, Carracedo A, Lareu M and McNevin, D: A SNaPshot of next generation sequencing for forensic SNP analysis. Forensic Science International: Genetics. 2015; 14: 50-60.

133. NEXT GENERATION SEQUENCING OF REFERENCE SAMPLES WITH THE HID-ION AMPLISEQ™ IDENTITY PANEL USING SINGLE PUNCHES FROM FTA-CARDS

Buchard A., Kampmann M. L., Børsting C., Morling N.

Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Anders.buchard@sund.ku.dk

Next generation sequencing (NGS) offers the possibility of obtaining more information from a sample than obtained with capillary electrophoresis (CE) techniques. In addition to revealing the complete variation of STRs, it is possible to type STRs, SNPs, indels, etc. in the same reaction. However, before NGS is implemented in forensic case work, it is important that the technology is thoroughly validated and that solutions for high throughput typing of samples are made available.

Many forensic laboratories use various forms of filter paper for collection and storage of DNA. The Section of Forensic Genetics, University of Copenhagen generally STR type DNA from reference samples deposited on Whatman™ FTA-cards with mouth swabs allowing for direct PCR and high throughput STR typing using PCR-CE. The HID-Ion Ampliseq™ Identity Panel amplifies 124 SNPs in one multiplex PCR. The products are sequenced using the Ion Personal Genome Machine (Ion PGM™). We typed 40 reference samples with the HID-Ion Ampliseq™ Identity Panel using washed 1.2 mm punches from FTA-cards and compared the results to those obtained with EZ1 extracted DNA. The data

showed that single punches of FTA-cards are well suited for NGS with the HID-Ion Ampliseq™ Identity Panel. This allows for simple NGS investigations of reference samples.

134. HIGH-THROUGHPUT SEQUENCING OF FORENSIC STRS AND SNPS USING THE MISEQ BENCHTOP SEQUENCER

Xavier C.[1], Parson W.[1,2]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[2] *Forensic Science Program, The Pennsylvania State University, PA, USA*

catarina.gomes@i-med.ac.at

Next Generation Sequencing (NGS) technologies are continuously increasing their impact on forensic genetics. The multiplexing capacity and ultimate sequencing resolution of these technologies bring a new perspective to the field, allowing the assembly of different markers in one reaction and the characterization of intra-allele variation. This is especially relevant in STRs since it allows the deconvolution of isometric alleles, leading to the description of new alleles and a better analysis of mixtures, therefore increasing discrimination power and consequently weight of evidence. The empowered data generation per run will revolutionize databasing expansion and might in long term not only help reaching uniformity but also lower the costs. Several assays comprising different forensic markers have been proved successful for future applications, however the absence of a commercial routine oriented kit, assembling different markers as STRs and SNPs, was noted. In this context Illumina creates the ForenSeq Signature DNA Prep Kit as the new high-throughput kit for forensic analysis, which combines a set of 59 STRs (autosomal, X and Y-STRs) and up to 172 SNPs for identification, phenotyping and ancestry information retrieving purposes. Here we analyzed the beta version of ForenSeq by performing a series of different assays as reproducibility, sensitivity, mixture, concordance and casework type samples, where we observed very promising results. This study explores a method that is a potential candidate to allow a smooth transition into NGS technologies, and in future help forensic genetic laboratories to improve their capacities both in casework analysis resolution, as well as in databasing competence.

135. RECOVERING GENOMIC AND WHOLE MITOCHONDRIAL PROFILES FROM SINGLE HAIR ROOTS WITH QPCR AND NEXT GENERATION SEQUENCING

Chang J.[1], Olson S.[1], Lagace R.[1]

[1] *Thermo Fisher Scientific Inc., South San Francisco, CA U.S.A.*

Joseph.Chang@thermofisher.com

Hair roots are notorious of providing limited amounts of genomic DNA from casework samples. Analysts will more often than not, skip post-extraction quantification knowing that there are insufficient quantities to report a full Short Tandem Repeat (STR) profile from current Capillary Electrophoresis (CE) technologies. However, the QuantiFiler® Trio DNA Quantification Kit is able to successfully detect and qualify picogram levels of genomic DNA extracted from <5mm hair roots. At this stage, analysts are able to accurately calculate input quantities for downstream processing. However, despite having accurate DNA quants, an incomplete STR profile will be generated for compromised hair samples. A modified PrepFiler *Express* BTA™ protocol was thus developed that not only captures genomic material, but also mitochondrial DNA. Though it is possible to create mitochondrial profiles through Sanger sequencing, it is very time consuming, labor intensive, and solely targets to the Hypervariable Regions (HV1 & HVII). Therefore, a short amplicon (~175 base pairs) whole mitochondrial tiling path was developed for the Next Generation Sequencing (NGS) Ion Personal Genome Machine (PGM™) System to facilitate sequencing the entire mitochondrial genome of highly degraded samples. This short amplicon design increases discrimination of compromised samples outside of the HV Regions. The HID-Ion AmpilSeq™ Ancestry and Identity Panels were

both used, in tandem with the mitochondrial panel, to target the genomic DNA and serve as a complement to incomplete CE STR profiles. Hair roots and buccal samples were processed through the PGM™ system, and the results compared to current CE STR results.

136. DEVELOPMENT AND VALIDATION OF A TARGETED NEXT GENERATION SEQUENCING SOLUTION FOR FORENSIC GENOMICS

Guzman E., Varlaro J., Stephens K., Holt C. L.

Illumina, San Diego, CA, USA

Sequencing (NGS) by Synthesis (SBS) enables the entire human genome to be sequenced in one day. As a simpler yet highly effective alternative, forensic scientists can choose to perform targeted sequencing of PCR products. By sequencing a dense set of forensic loci, casework and database efforts are directed toward the genomic regions that best answer forensic questions, relieving privacy concerns and simplifying analysis. Because it does not depend on allele separation by size, the number of targets interrogated is not limited, allowing a more comprehensive result to be generated.

We describe the development and validation of a targeted amplicon panel and associated bioinformatics tools for forensic genomics that combines and interrogates a core of global short tandem repeat markers used routinely today, along with additional forensic loci that can provide information when standard markers would fail to sufficiently resolve a case. Maximizing the number and types of markers that are analyzed for each sample provides more comprehensive and discriminating information for standard samples, as well as challenging samples that contain low quantities of DNA, degraded and/or inhibited DNA, and complex mixtures. The targeted amplicon panel will enable more complex kinship analysis to be performed, and can also reveal phenotypic and biogeographical ancestry information about a perpetrator to assist with criminal investigations. This capability is expected to dramatically improve the ability to investigate dead end cases, where a suspect reference sample or database hit are not available. We will describe the complete workflow, system, and data analysis tools, and present data from validation and collaborator studies including reproducibility, sensitivity, actual forensic samples, and concordance with standard capillary electrophoresis methods.

137. DEVELOPMENT OF A DNA MIXTURE DECONVOLUTION SOLUTION FOR FORENSIC EVIDENCE USING MASSIVELY PARALLEL SEQUENCING

Cavanaugh S.[1], Kennedy K.[1], Feldman A.[2], Lin J.[2], Becker J.[2], Slack D.[1], Davoren J.[1]

[1] The Bode Technology Group, Inc., Lorton, USA
[2] NexGen Forensics, LLC

Jonathan.davoren@bodetech.com

With rapidly improving chemistries and decreasing cost, massively parallel Sequencing has incredible potential for forensic investigations. Sequencing STRs can overcome some of the limitations of genotyping by capillary electrophoresis and provides increased statistical significance with backwards compatibility to size based methodologies. The additional information provided by sequencing can be invaluable for the deconvolution of complex forensic mixtures. Under a United States Government funded effort, The Bode Technology Group, Inc. (Bode) in collaboration with NexGen Forensics, LLC (NexGen) have developed a full solutions pipeline to allow for the easy data interpretation of complex DNA mixtures using the commercially available Illumina ForenSeq™ DNA signature prep kit chemistry. This pipeline not only includes laboratory processes, but also the development of a software algorithm and Graphical User Interface (GUI) that allows for the analysis of sequenced forensic Short Tandem Repeat (STR) markers from complex human DNA mixtures. The software is capable of analyzing sequence variants found within the STR, and also from flanking regions to add statistical power

to forensic likelihood ratios and paternity indices. Additionally, the solution provides a putative DNA profile from complex DNA mixtures to allow for easy database query and investigative lead generation. While the current solution uses Illumina chemistry and the MiSeq desktop sequencer, the ultimate goal of this effort is a platform and chemistry agnostic system capable of analyzing and deconvoluting complex mixtures obtained by any massively parallel sequencing technology.

138. THE STUDY OF A PANEL CONSISTING OF STRS,SNPS AND INDELS ON ILLUMINA MI-SEQ PLATFORM

Su Q.[1], Jin B.[1,3], Mao J.[2], Zhu J.[1], Bai X.[2], Wang L.[1], Jiang Y.[1], Peng D.[1], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China
[3] Department of Forensic Medicine, North Sichuan Medical College, Nanchong 637000, PR China

liangweibo@scu.edu.cn

STRs, SNPs and Indels have their respective chracteristics that make them ideal markers for forensic application. Although STRs have been proved comparatively perfect with the limitation of stutter products, the extra information from SNPs and Indels is still essential for forensic purposes, such as the ancestry-informative markers, forensic DNA phenotyping markers. As we all know, analysis of those markers traditionally profiled through capillary electrophoresis(CE) platform after PCR. However, CE-based method has some limitations, such as the limited number of loci and the method of SNP profiling is different with STR. Technically, the Next Generation Sequencing(NGS) has been considered a viable method recently allowing high-throughput coverage at a relatively reasonable price for routine implementation in forensic genetics. NGS offers several advantages over CE, such as virtually unlimited multiplex of loci, combining both STR, SNP and Indels. We have planed to set up a panel which could be analyzed on Illumina Mi-Seq platform. The panel consists of STRs, SNPs and Indels which can offer the identification power, the ancestry informations, and some sepcial information from Y chromosomes. The genotypes of those markers can be obtained by a single NGS. The panel includes more than 50 SNPs, 13 autosomal STRs, 15 Y STRs and 15 Indels. 24 samples were analyzed in one sequencing. And the data was typed by Forensic-Loci-finder which was introduced in our another abstract. The pre-results show that only partial profiles of those loci are obtained. It suggests that the proceduer of purification and multiple PCR need to be improved.

139. STR GENOTYPING WITH ION TORRENT PGM AND STR 10-PLEX SYSTEM: HIGHLIGHTS ON PERFORMANCE AND DATA INTERPRETATION

Bottino C. G.[1,2], Budowle B.[3,4], King J.[3], Churchill J.[3], Silva R.[5], Moura-Neto R. S.[2,6]

[1] Instituto de Pesquisa e Perícias em Genética Forense, DGPTC/PCERJ, Rio de Janeiro, Brazil
[2] Instituto Nacional de Metrologia, Qualidade e Tecnologia, Duque de Caxias, Brazil
[3] Institute of Applied Genetics, Department of Forensic and Investigative Genetics, University of North Texas Health Science Center, Forth Worth, Texas, USA
[4] Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia
[5] Instituto de Biofísica Carlos Chagas Filho/UFRJ, Rio de Janeiro, Brazil
[6] Instituto de Biologia/UFRJ, Rio de Janeiro, Brazil

carolina.bottino.bio@gmail.com

Massively parallel sequencing (MPS) platforms allow the simultaneous analysis of thousands to millions of DNA fragments, generating large amounts of data in a relatively short time frame compared with traditional sequencing methods. Recently, it has been demonstrated that MPS platforms may be

applicable in a forensic context, both for STR and SNP genotyping. Before a new technique is incorporated into routine casework, it must be extensively studied and validated. The aim of this study is to examine parameters for data interpretation generated for STR genotyping in a MPS platform. Reference DNA (1.0 and 2.0 ng/uL) was amplified with Ion Torrent HID STR 10-plex kit™ (Thermo Fisher) and sequenced in Ion Torrent PGM™ platform (Thermo Fisher). Parameters evaluated included percent loading, live isps, depth of coverage; allele coverage ratios; stutter ratio; noise (i.e., sequence coverage ratio); and sequence variants. For 1.0 ng/uL input DNA, number of reads ranged from 558 to 4916 per locus; artifacts ratio ranged from 0.046 to 0.129 and noise ratio varied from 0.089 to 0.24. For 2.0 ng/uL input DNA, parameters were similar, although slightly poorer (422-4394 of read number; 0.073 – 0.113 artifact ratio and 0.104 – 0.346 noise ratio). Allele dropout was observed at locus D8S1179, for both concentrations, and at locus vWA only for 2.0 ng/uL. Alleles from loci D3S1358 and vWA demonstrated intra-allelic variation. These data indicate that MPS could be very useful for STR genotyping, improving accuracy and mixture interpretation. We thank Thermo Fisher for proving the Ion Torrent™ HID STR 10-plex kit.

1. Seo SB, King JL, Warshauer DH, Davis CP, Ge J, Budowle B: Single nucleotide polymorphism typing with massively parallel sequencing for human identification. International Journal of Legal Medicine. 2013; 127(6) : page 1079 – page 1086
2. Fordyce SL, Mogensen HS, Børsting C, Lagacé RE, Chang C, Rajagopalan N, Morling N: Second-generation sequencing of forensic STRs using the Ion Torrent™ HID STR 10-plex ant the Ion PGM™. Forensic Science International:Genetics. 2015; 14 : page 132 – page 140
3. Associação Brasileira de Normas Técnicas: NBR ISO/IEC 17.02.. Rio de Janeiro: 2005, 29 p.

140. HUMAN IDENTIFICATION OF 120 SNP WITH PGM SEQUENCER SYSTEM

Zhang S., Bian Y., Wang Z., Li C.

Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, P.R. China, Shanghai, China

zsh-daisy@163.com

Single nucleotide polymorphisms (SNPs) provide a variety of new, important information for human identification. And SNPs enable high recovery of information from degraded samples (such as in the case of mass disaster victims). The ability to multiplex hundreds to thousands of SNP primers, and concurrently amplify and sequence the SNPs, provides significant convenience and high discrimination power. With the bench-top Sequencer of Personal Genome Machine™ (PGM™), the HID-Ion AmpliSeq™ Identity Panel, a primer pool of 90 autosomal SNPs (43 Ken Kidd SNPs and 48 SNPforID, with 1 shared SNP) and 30 upper Y-clade SNPs for human identification, was evaluated in this study. qPCR method with Ion Library Quantitation kit was applied for the DNA library quantification. Concordance study was performed with two control samples of 9947A and 9948 between the HID-Ion ampliseq SNP assay and Sanger sequencing. Full concordance were obtained except for genotype of rs576261 and rs214955 with allelic imbalance by NGS Sequencing. By studying the HID-Ion ampliseq panel in 45 individuals, SNPs of rs214955, rs4530059, rs2342747, rs1523537, rs576261 and rs12997453 were recognized as poorly performing loci, either with allelic imbalance or with lower coverage (<100 Reads). Sensitivity testing demonstrated that with DNA range from 10ng-0.5ng, all correct genotype at the 120 SNPs were detected. However, when DNA lower than 1ng, some SNPs loci showed allelic imbalance. Overall, this study showed that genotyping a battery of the 120 SNPs, satisfying the need for human identification in Chinese Han population, is feasible with next generation sequencing of Ion PGM Sequencer.

141. PILOT STUDY OF 124 SNP INCLUDED IN HID-ION AMPLISEQ PANEL IN A FOUR-GENERATION FAMILY

Zhang S., Wang Z., Bian Y., Li C.

Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, P.R. China, Shanghai, China

zsh-daisy@163.com

Next generation sequencing (NGS), providing more comprehensive and intensive studies of genetic markers, are highly interesting for forensic genetic community. Ion Personal Genome Machine (PGM) (Life Technologies), which combines semiconductor sequencing technology with natural biochemistry to directly translate chemical information into digital data, was adopted as the studied bench-scale NGS platform. HID-Ion ampliseq™ SNP-124 panel, a primer pool of 90 autosomal SNPs and 34 upper Y-clade SNPs for human identification, was evaluated on the PGM Sequencer System in our previous study. DNA above 1 ng can obtain ideal results (optimal allelic balance and high coverage). For the forensic efficiency of the 124 SNPs in Chinese Han population, no linkage disequilibrium (LD) was existed among the 90 auto-SNPs with TDP (Total discrimination power) was 1-5.2192E-23 and 6 haplotypes were observed of the 34 Y-SNPs. 10 ng Initial DNA of 15 individuals included in the four-generation family were studied with the HID-Ion ampliseq™ SNP-124 panel in this study. Among the four-generation family, obtained genotyping results follow the Mendel' law. 8 types of relationships (Unrelated, Parent-Child, Grandfather-Grandchild, Great grandfather-Great grandchild, Full-Sibling, Uncle/Aunt-Niece/Nephew, Cousins and Grandchild-Grandfather'brother) were observed in the family. Parameter of IBS (identity by state), a term used in genetics to describe two identical alleles or two identical segments or sequences of DNA, was applied in the analysis. Results shows that Full-Sibling and Parent-Child enjoy the highest IBS score while unrelated individuals enjoy the lowest IBS score.

142. GENOTYPING 27 BONES USING ILLUMINA FORENSEQ DNA SIGNATURE KIT

Gréen H.[1,2], Grandell I.[1], Tillmar A.[1]

[1] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden
[2] Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden

Henrik.green@rmv.se

The field of kinship analysis and forensic case work is moving into the field of next generation sequencing. The aim of this study was to investigate if we could use the Illumina ForenSeq DNA signature kit to genotype autosomal STRs, X and Y STRs as well as SNPs in bone samples for use in disaster victim identification and kinship analysis. The beta-version of the ForenSeq kit was used to genotype 18 bone samples that previously had given full profiles and 9 the resulted in partial profiles using capillary electrophoresis (CE). For the samples with full profiles in the CE we got on average 45 out of 63 (81%) STR markers per sample that passed the QC in the software. Some markers were blank in all samples maybe due to absence of specific primers in the beta-test. Heterozygote balance were on average 82% (stdev=11%) and mean coverage was 2370X per marker. 94% of the 95 SNPs passed QC and the allelic balance were 0.50 (stdev=7%). Mean coverage was 950X per SNP. Two of the samples showed disconcordance between the CE and NGS results, one due to extreme 260/230 ratio (4.7) and one due to drop-out. For the samples with partial profiles 47% of the SNPs passed QC on average with a mean coverage181X. 33% of the STR markers passed QC with a mean coverage of 486X. The beta-test worked rather well for a beta-test, but two independent runs will be required. SNPs had a higher success rate especially for degraded samples.

143. FORENSIC GENETICS IN NGS ERA: NEW FRONTIERS FOR MASSIVELY PARALLEL TYPING

Iozzi S., Carboni I., Nutini A. L., Ricci U., Contini E.,
Pescucci C., Frusconi S., Torricelli F.

Diagnostic Genetics Unit, AOU Careggi, Florence, Italy

ricciu@aou-careggi.toscana.it

Next Generation Sequencing (NGS) represents the newest approach for massive DNA sequencing. Although this innovative approach is a well-established point in diagnostic genetics, the applicability in forensic field is still limited to sporadic pilot studies. This limit arises from the samples' complexity, in terms of poor quality/quantity ratio, leading to a difficult setting in analytic systems.

In this study, first NGS forensic data with a new analytic protocol are presented; data were obtained with a pre-release version of the MiSeq system (Illumina). The system is able to sequence, at the same time, a wide panel including autosomal 63 STRs and 95 SNPs commonly used in forensic practice.

In the first part of the study sensitivity, reproducibility and concordance studies were performed by using DNA controls with known genetic profile; also mixtures with different male/female concentrations were analysed, in order to verify the panel performance in comparison with traditional kits.

In the second part of the experiment, 60 casework samples coming from different tissues, biological fluids, traces on supports, lysates, different aged/degraded samples were analysed. Bioinformatic analysis was performed with MiSeq FGx System BETA version (Illumina), an innovative software with an easy interface that permits a rapid data interpretation by filtering the big data quantity generated by the instrument.

The comparison between new data and genetic profiles obtained with traditional typing on the same samples, the sensitivity and the potential of NGS approach represents an interesting starting point in introducing this innovative approach on forensic genetics scenario.

144. MODELLING NOISE IN SECOND GENERATION SEQUENCING FORENSIC GENETICS STR DATA USING A ONE-INFLATED (ZERO-TRUNCATED) NEGATIVE BINOMIAL MODEL

Vilsen S. B.[1], Tvedebrink T.[1], Morgensen H. S.[2], Morling N.[2]

[1] Department of Mathematical Sciences, University of Aalborg, Denmark
[2] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

svilsen@math.aau.dk

We present a model fitting the distribution of non-systematic errors in STR analysis using second generation sequencing (SGS). The model fits the distribution of non-systematic errors, i.e. the noise, using an one-inflated, zero-truncated, negative binomial model. The model is a two component model. The first component models the excess of singleton reads, while the second component models the remainder of the errors according to a negative binomial distribution.

We estimate the parameters of this model in two ways: (1) We maximise the likelihood using a gradient function and (2), we use the expectation-maximization (EM) algorithm. The estimated parameters are used to create dynamic, sample specific thresholds for noise removal using locus specific proportions of the negative binomial distribution.

Based on data from dilution series experiments (amounts of DNA ranging from 100pg to 2ng) conducted at The Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, the method was compared to that of a naïve model that implies the removal of reads with a coverage of less than e.g. 5-10% of the total locus coverage. In comparison, our method resulted in three allelic drop-outs (true alleles below threshold), whereas the 10%-threshold induced 12 drop-outs. The non-filtered error reads (e.g. stutters, shoulders and reads with miscalled bases) will subsequently be modelled by different statistical methodologies.

145. SEQUENCE-BASED ANALYSIS OF STUTTER AT STR LOCI: CHARACTERIZATION AND UTILITY

Aponte R. A.[1], Gettings K. B.[2], Duewer D. L.[2], Coble M. D.[2], Vallone P. M.[2]

[1] Department of Forensic Sciences, The George Washington University, Washington, DC, U.S.
[2] National Institute of Standards and Technology, Gaithersburg, MD, U.S.

katherine.gettings@nist.gov

The implementation of next generation sequencing (NGS) technologies creates the potential for changing the method by which the forensic science community genotypes short tandem repeat (STR) loci. However, transitioning to a new technology would require a new understanding of artifacts that may arise with the use of NGS. Stutter has been well characterized for CE technologies; however, NGS workflows may use different polymerases and amplification approaches, which could alter the characteristics of this artifact. For tetranucleotide repeat motifs stutter is most commonly seen in the n-4 position in CE data, but may be observed more rarely in the n+4 and n-8 positions. From our NGS data we frequently observe detectable sequences consistent with stutter at the n+4 and n-8 positions, and even detect stutter at the n-12 position at some loci. It is possible that these alternate types of stutter events occur at similar levels in CE workflows and go undetected due to the analytical threshold employed or because the artifacts do not exceed the baseline signal. Comparing stutter events in NGS data to what has been observed by CE will improve our understanding of the effects of library preparation and sequencing. Characterizing stutter events by sequence will contribute to the development of interpretation guidelines and facilitate implementation of NGS technology. Further, determining stutter ratios for each isoallele (alleles that are of the same length but different sequence) would allow for individual sequence thresholds to be set, which could then be used to improve mixture interpretation models.

146. NEXT GENERATION SEQUENCING AS AN ALTERNATIVE METHOD OF CLONING PCR PRODUCTS FOR ANCIENT DNA AUTHENTICATION

Palencia-Madrid L., de Pancorbo M. M.

Universidad del Pais Vasco, Spain

Ancient DNA studies require the authentication of the results obtained since it is essential to verify the absence of contaminating sequences on them. Cloning the amplified products obtained from these samples has been one of the principal methods traditionally used to assure the absence of contamination. However, this is an arduous analysis, not routinely applied in forensic laboratories. Recently, the development of new sequencing technologies opens new ways to authenticate ancient DNA results. Data obtained by next generation sequencing allow the discrimination between endogenous and contaminating sequences.

Here we present the results obtained by both traditional cloning and next generation sequencing (NGS) based on ion semiconductor sequencing on Ion Torrent™ PGM®. These analyses were performed to verify the Sanger sequencing results of some ancient samples. The comparison of both methods shows that ion semiconductor sequencing provides a better estimation on the proportion of contaminating sequences present in the sample and more information about the preservation state of the analyzed DNA than traditional cloning. Thus, NGS technology, and in particular, ion semiconductor sequencing, is revealed as a suitable method to authenticate the results obtained from degraded and ancient DNA samples, since it provides more information, it is less time and money consuming and it would be unaffordable to obtain the same quantity of data by traditional cloning.

Theme 06: New Technologies

148. RAPID DNA MATURITY ASSESSMENT

Romsos E. L.[1], Lembirick S.[2], Vallone P. M.[1]

[1] National Institute of Standards and Technology, Gaithersburg, MD, U.S.
[2] Montgomery College, Rockville, MD, U.S.

erica.romsos@nist.gov

Integration of the extraction, amplification, separation, and detection processes for forensic DNA typing is a challenging goal. Several parallel efforts have been made to integrate the forensic workflow and utilize a simple swab in, answer out process within a single platform [1-3]. Of the multiple efforts, two platforms were tested as a part of a rapid DNA maturity assessment in 2014. The assessment was conducted with sets of blinded single-source reference samples to gauge the typing success of the current rapid DNA typing technology. Samples were provided to participants for testing on the individual rapid platforms, and data was returned to the National Institute of Standards and Technology (NIST) for review and analysis. Both automated and manual review of the data sets were conducted to assess the success of typing the CODIS core loci. Genotyping profiles from the multiple platforms, participants, and STR typing chemistry was combined into a single analysis to assess the current maturity of Rapid DNA technology. The presented results will focus on genotyping success rate, peak height ratios, and stutter artifacts across two platforms and multiple STR kit chemistries.

References

1. Tan E, Turingan RS, Hogan C, Vasantgadkar S, Palombo L, Schumm JW, et al. Fully integrated, fully automated generation of short tandem repeat profiles. Investig. Genet. 4 (2013) 2041-2223.
2. Jovanovich, S., Bogdan, G., Belcinski, R., Buscaino, J., Burgi, D., Butts, E. L. R., et al. Developmental validation of a fully integrated sample-to-profile rapid human identification system for processing single-source reference buccal samples. Forensic Sci. Int. Genet. 16 (2015) 181-194.
3. Hennessy LK, Mehendale N, Chear K, Jovanovich S, Williams S, Park C, et al. Developmental validation of the GlobalFiler express kit, a 24-marker STR assay, on the RapidHIT System. Forensic Sci. Int. Genet. 13 (2014) 247-258.

149. RETROTRANSPOSABLE ELEMENTS: NOVEL AND SENSITIVE DNA MARKERS AND THEIR APPLICATION IN HUMAN IDENTITY

Sinha S., Murphy G., Brown H., Green A., Montgomery A., Carrol M.

InnoGenomics Technologies; 1441 Canal Street, Suite 307; New Orleans, LA 70112, USA

ssinha@innogenomics.com

Retrotransposable elements (REs) consisting of long and short interspersed nuclear elements (LINEs and SINEs) are useful for human identity as well as bio-ancestry testing. Until now, however, due to the inherent size difference (>300bp) associated with insertion and null alleles (or INNULs), the use of REs has not been practical for human identity applications. To circumvent the allele size disparity, we have developed a novel primer design methodology that reduces the overall amplicon size and enables the development of 20 RE's and Amelogenin into a single multiplexed typing system, InnoTyper™ 21, that is fully compatible with current capillary electrophoresis platforms. All markers of the InnoTyper™ 21 system are less than 124 bp, and data will be presented showing utility for analyzing highly compromised single source degraded DNA samples, including old degraded bones and 2 cm cut hairs without roots. These 21 InnoTyper™ markers can also be combined with ancestry informative ALU (AI-ALU) markers, all of small amplicon size of less than 100 bp, to provide additional identity information. We have selected 30 AI-ALUs most informative for bio-ancestral identification from the ALUs identified and curated by

the 1000 Genome Project Structural Variations Group. These AI-ALUs were selected from the data set of 2504 unrelated individuals from 26 populations and therefore provide significant resolution of biogeographical origin. The development of a small amplicon, multiplexed primer kit for preparing next-generation sequencing (NGS) libraries that are useful for forensic and bio-ancestral identification from challenging DNA samples will be discussed.

150. DEPARRAY DIGITAL SORTING OF BIOLOGICAL MIXTURES ACHIEVES ISOLATION OF 100%-PURE CELL POPULATIONS FOR CLEAR-CUT GENETIC ANALYSIS

Fontana F.[1], Rapone C.[2], Bregola G.[1], Aversa R.[3], Lanzellotto R.[1], Medoro G.[1], Manaresi N.[1], Vittorioso P.[3], Berti A.[2]

[1] Silicon Biosystems S.p.A., Bologna, Italy
[2] Reparto Investigazioni Scientifiche Carabinieri R.I.S., Roma, Italy
[3] Dipartimento di Biologia e Biotecnologie "C. Darwin", Sapienza Università di Roma, Roma, Italy

ffontana@siliconbiosystems.com

Background:

Biological mixtures represent a relevant number of all samples reaching the forensic the forensic labs. DNA profiling of these mixtures is hampered by the contributions of different individuals. In principle, when different individuals contribute with different cell types, their genetic profiles may be obtained by cell separation. However, no purification method reported to date can guarantee an isolation of pure cells belonging to the same class (e.g. epithelial, blood cells, sperm).

Here we show for the first time how the use of DEPArray™, an image-based, microfluidic, digital sorter with single-cell resolution [1.2], can provide 100%-pure cells for exact genetic profiling.

Materials and Methods:

To simulate a forensic evidence, few microliters [1,5-75 µl] of peripheral blood, semen and saliva from different donors were mixed in different ratios, spotted on fabric, paper or swab and let dry. After storage (1-42 days, mean 17,5), simulated evidences were resuspended, immunofluorescently labeled and loaded on DEPArray™ for sorting and isolation. AmpFlSTR®NGM Select Kit was used to profile recovered cells, single or pools.

Results:

We demonstrated the ability to reliably:

1. Discriminate between cell types in the mixture (sperm, epithelial or blood cells).
2. Isolate, from the same biological mixture, multiple aliquots of pure cells (1-50) of each type.
3. Obtain complete, clean genetic profiles from as low as 5 homogeneous cells for each donor in the mixture, without detectable allelic contamination.

Highlights:

DEPArray™ Technology can resolve the analysis of biological mixtures, through precise sorting of different cell types, achieving 100%-pure genetic profiles.

References

1. Polzer et al. Molecular profiling of single circulating tumor cells with diagnostic intention. EMBO Mol Med. 2014 Oct 30;6(11):1371-86.
2. Manaresi et al. A CMOS Chip for Individual Cell Manipulation and Detection. IEEE Journal of Solid State Circuits, vol 38, n. 12 Dec. 2003

151. A NEW APPROACH FOR THE SEPARATION OF SPERMATOZOA FROM OTHER CELL TYPES IN FORENSICALLY RELEVANT SAMPLES

Grosjean F.[1], Castella V.[3]

[1] Forensic Genetics Unit, University Hospital of Lausanne, Switzerland

Frederic.Grosjean@chuv.ch

Several methods are available to separate spermatozoa from other cell types, mainly epithelial cells, from samples collected following a sexual assault. We set up a new, fast, procedure for the separation of cells from cotton swabs, or other supports, with little risk of inter-sample contamination. Based on antibodies, this procedure was first developed, and optimized, by flow cytometry using fresh cellular mixtures. It allows the recovery of two enriched cell fractions: a spermatic fraction, generally enriched with the alleged offender's spermatozoa, and a non-spermatic fraction, generally enriched with cells from the victim.

Subsequently, processing of six month old mock samples, made of buccal swabs and sperm dilutions, resulted in full single NGM Select DNA profiles of the sperm donor for the spermatic fractions, and single NGM Select DNA profiles of the victims for the non-spermatic fractions. Untreated duplicate samples processed in parallel only provided the autosomal DNA profiles of the victims.

The separation can be performed in less than 90 minutes; combining recovery and separation buffers with commercially available material, this new procedure can be rapidly tested and adopted by forensic laboratories worldwide. Moreover it has potential for automation, and therefore could provide a mean to reduce existing backlogs rapidly.

152. NEW Y-SCREEN ASSAY USING THE QUANTIFILER TRIO DNA QUANTIFICATION KIT

Holt A.[1], Olson S.[1]

[1] *Thermo Fisher Scientific, U.S.A.*

allison.holt@thermofisher.com

Sexual Assault Kit (SAK) samples are among the most difficult sample types encountered by many forensic laboratories. Differential extraction procedures used as part of a sexual assault workflow are both time consuming and labor intensive. For many laboratories, SAKs represent a high percentage of current and backlogged cases and often yield no presence of male DNA, when analyzed downstream by autosomal and Y STR amplification. To assist in the decision making process of whether to take a sample forward to differential extraction, we have developed a novel DNA screening workflow allowing customers to quickly assess whether swab evidence from an SAK contains a male contributor prior to the standard labor-intensive differential extraction procedures used in forensic laboratories. This Y-screen assay starts with a small cutting of an SAK swab placed into a buffer which lyses cells (including sperm) in only 10 minutes. This is immediately followed by a quick neutralization step and dilution before addition to the Quantifiler® Trio assay. We demonstrate that the sensitivity of the assay correlates to the results obtained from differential extraction procedures with our studies and STR analysis, as well as the results from forensic laboratories using a range of sample types, differential extraction procedures and STR chemistries. We show that this technique can provide a complementary DNA confirmative assay to complement the current presumptive screening techniques commonly used by labs. This Y-screen assay solves important sample screening and processing problems, allowing forensic laboratories to more rapidly process SAK samples and therefore help to assist in decreasing overall SAK turnaround times.

153. VALIDATION OF A RAPID DNA PROCESS USING A PLATFORM OPTIMIZED FOR BOOKING STATION ENVIRONMENTS

Barican A., Buscaino J., Chear K., Jovanovich S., Klevenberg J., King D., Lin F., Nguyen P., Park C., Pearson F., Porporato R., Salodkar S., Scheuren R., Smith C., Tsou D., Vangbo M., Wunderle J.

IntegenX Inc., Pleasanton, USA

stevanj@integenx.com

IntegenX has developed a next generation of Rapid DNA instrument RapidHIT® ID, specifically for the needs of booking stations. The FBI vision for Rapid DNA is to collect and test samples at time of arrest. With the introduction of the Rapid DNA Identification Act of 2015 in Congress and the Md. v. King decision, the overall judicial and legislative blockages are now being cleared at the Federal level in the USA. Quality assurance standards are being worked on and the plan for incorporation of DNA information into the NGI system is underway. RapidHIT ID processes reference samples and generates real time profiles in less than 90 min using a lower-cost, single-use consumable in accordance with Rapid DNA analysis process. Bulk reagents and the capillary electrophoresis module are replaced in a 250-use cartridge. RapidHIT ID uses NDIS approved chemistry and a single capillary analysis system with laser induced fluorescence detection. An NDIS approved expert system analyzes the profile and produces CODIS compatible and rCMF files. The system includes a built in fingerprint reader and camera for access and documentation. RapidHIT ID will be described and accuracy, inhibition, precision, sensitivity and mixture studies will be presented. The state of Rapid DNA technology and implementation in booking stations will be discussed.

154. ANHYDROBIOSIS AS AN ALTERNATIVE TO FREEZING FOR THE PRESERVATION OF EXTRACTED DNA. COMPARAISON OF QIASAFE AND DNASTABLE LD

Frippiat C.[1]

[1] *National Institute of Criminalistic and Criminology, Brussel, Belgium*

Christophe.frippiat@just.fgov.be

Success of forensic genetics has led to a considerable number of samples to store. Preserving the integrity of forensic samples is of first importance. Retesting of the samples should be guaranteed for years. Usually the storage of DNA is freezing at -20°C. New methodology allowing the storage of DNA at room temperature was recently developed. This technology is based on anhydrobiosis and allows room temperature storage of DNA. Our previous results, using Qiasafe (Qiagen) to protect DNA, show that after 2 years of ageing at room temperature, there is a decrease of the number of observed alleles and of peak heights. In the view of these results, we tested DNAStable LD (Biomatrica) in comparison with the Qiasafe.

Using the Qiasafe and DNAStable LD, we compared the quantity and the quality of recovered DNA after anhydrobiosis or -20°C storage, by performing STR profiling after short-term storage of low concentrations of one contributor DNA. We also evaluated the effect of multiple cycles of hydration/dehydration.

Finally, we evaluated the ability of this technology to preserve DNA samples mimicking true mixture casework samples for long periods. For this, we used accelerated ageing by heating to reach an ageing of 15 years and low concentrations (15pg/µl) of mixed DNA. The quality of the storage was evaluated by quantifying peak heights from STR's genotyping and the number of observed alleles.

155. MICROHAPLOTYPE: A POTENTIAL GENETIC MARKER TO SUPPLEMENT THE STANDARD CODIS PANEL

Jing Zhu J.[1], Nan Zhou N.[3,4], Jiang Y.[1], Wang L.[1], He W.[1], Peng D.[1], Su Q.[1], Mao J.[2], Chen D.[2], Liang W.[1], Zhang L.[1]

[1] *Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China*
[2] *Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China*
[3] *College of life Sciences, Sichuan University, Chengdu 610041, Sichuan, China*
[4] *Bio-resources Key Laboratory of Minister of Education, Sichuan University, Chengdu 610041, Sichuan, China*

liangweibo@scu.edu.cn

Microhaplotypes which introduced in another abstract have higher

heterozygosity than SNPs and are globally informative, and mutation have not been found in father-son pairs yet. Microhaplotypes shows the potential for personal identification and parentage testing. The microhaplotype panel need to be established which could approaching the power of STRs. Therefore, more microhaplotypes with suitable polymorphism are needed. In this study, 44 microhaplotype loci were screened out following the principle mentioned in another abstract from online database. After analyzing the PCR results of 44 loci by polyacrylamide gel electrophoresis (PAGE), 25 loci were chosen to do the further analyses. DNA of 18 independent random individuals and 6 father/mother-son pair family were extracted. 5 multiplex of the 25 microhaplotypes were designed, and amplicons were sequenced by illumina Mi-seq platform after purified and labeled through the commercial kits. Forensic-Loci-finder(FLfinder) was used to analyzing the FASTQ data and typing the microhaplotypes. 6 of 25 loci showing the heterozygosity higher than 0.70, while 4 of the rest are between 0.40 to 0.70 and 3 loci didn't have more than one genotype. The panel consist of those 10 microhaplotypes have the TDP of 0.999996373, which is still lower than STR panels. The results shows that at least a 17 microhaplotypes panel may have the similar power with the STRs.

156. DNA BIOSENSOR AS A CONFIRMATORY TEST: STUDIES OF HOUSEHOLD CLEANERS EFFECTS OF ONTO ELECTROCHEMICAL DNA DETECTION

Oliveira N.[1], Campos-Ferreira D.[1], Bezerra W.[1], Zanforlin D.[1], Nascimento G.[1], Santos S.[2], Lima-Filho J.[1,3]

[1] Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife-Brazil
[2] Gerência Geral de Polícia Científica, Secretaria de Defesa Social do Estado de Pernambuco, Recife – Brazil
[3] Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife-Brazil.

nataliacybelle.89@gmail.com

The detection and identification of human body fluids at crime scenes is mainly made by colorimetric and crystallographic tests, which may not detect these samples when they are washed away. Currently, biosensors are emerging as alternative device in molecular biology, combining the specificity and sensitivity of biological systems with the computing power of microprocessors. Herein, we report a preliminary study of an electrochemical biosensor able to detect DNA sequences in solutions mixed with household cleaners, aiming to simulate the effects of these chemicals into sample detection. The device was made by pencil graphite, acting as a working electrode (PGE) and an Ag/AgCl pin as a reference electrode. Single-strand DNA probes (ssDNA) diluted in acetate buffer were immobilized on PGE surface, after applying a potential of +0.5 V. The hybridization assay was performed by immersing the ssDNA-PGE electrode in a buffer solution with the target sequence (100 nM) under specific annealing temperature for 10 min. Finally, to simulate the contamination of samples by household cleaners, dsDNA-PGE was immersed in solutions containing alcohol, bleach and detergent and stored at 27° C for 30 min. The current peaks obtained by ssDNA-PGE and dsDNA-PGE were highly distinguishable (118.6 nA and 24.435 nA, respectively), even when dsDNA-PGE was mixed with alcohol, detergent and bleach (35.81nA, 69.44 nA and 17.12 nA, respectively). These findings suggest that DNA biosensors have a great potential to be used as a confirmatory test to detect human body fluids in crime scenes.

1. Akutsu, T. et al. Comparison of automated and manual purification of total RNA for mRNA-based identification of body fluids., Forensic Science International. Genetics. 14 (2015) 11–7.
2. Virkler, K., Lednev, I.K. Analysis of body fluids for forensic purposes : From laboratory testing to non-destructive rapid confirmatory identification at a crime scene, Science. 188 (2009) 1–17.
3. Siddiquee, S. et al. Nanoparticle-enhanced electrochemical biosensor with DNA immobilization and hybridization of Trichoderma harzianum gene, Sensing and Bio-Sensing Research. (2014).
4. Campos-Ferreira, D.S. et al. Electrochemical DNA biosensor for human

papillomavirus 16 detection in real samples, Analytica Chimica Acta. 804 (2013) 258–263.

157. EVALUATION OF THE QUALITY OF STR PROFILES GENERATED BY MODIFIED RAPID DNA SYSTEMS

KitayamaT., Watahiki H., Mita Y., Fujii K., Nakahara H., Sekiguchi K.

National Research Institute of Police Science, Kashiwa, Japan

tetsushik@nrips.go.jp

The present study is aimed to evaluate the quality of STR profiles generated by two rapid DNA systems of DNAscan and RapidHIT. These systems can process buccal samples in a standard mode and other types of forensic samples in a recently developing low DNA mode. The 20 required and 3 recommended CODIS loci and other additional loci can be obtained by DNAscan 6C Rapid DNA Analysis System using PowerPlex Fusion 6C and by RapidHIT Human DNA Identification System using GlobalFiler Express.

Here we present the preliminary result of internal validations using the two instruments. These validations include reproducibility and precision, sensitivity and stochastic studies, mixture, and contamination assessment. We show the results of buccal samples, DNA swabbed samples, and the NIST SRM as reference samples and other low quality challenging samples such as saliva stains, bloodstains and hairs etc. We present the comparison of the results obtained by the conventional rapid DNA instruments with those obtained by the conventional capillary systems of 3130xl and 3500xL analyzers. As data analysis software, we use GeneMapper ID, GeneMapper ID-X, GeneMarker HID etc.

158. VALIDATION OF QUANTIFILER TRIO DNA QUANTIFICATION KIT IN FORENSIC SAMPLES

Gouveia N.[1,2], Brito P.[1,3], Serra A.[1], Balsa F.[1,3], Andrade L.[1,3], São Bento M.[1,3], Cunha P.[1], Bogas V.[1,3], Lopes V.[1,3], Porto M. J.[1,3]

[1] Forensics Genetic and Biology Service, Central Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal
[2] Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[3] CENCIFOR, Forensic Sciences Centre, Coimbra, Portugal

mariajoao.porto@dcinml.mj.pt

Forensic samples often have low quantity and/or degraded DNA, may contain PCR inhibitors, and, particularly in sexual assault samples, a high quantity of female DNA compared to male DNA. These factors can make it difficult to decide whether to continue with STR analysis, which STR kit to use and how much DNA to add to the reaction. The Quantifiler® Trio DNA Quantification kit helps overcome these obstacles through enhanced targets that have been designed to determine the quantity of DNA in highly compromised forensic samples, enabling to assess the level of DNA degradation which was not possible to achieve with the Quantifiler® Duo DNA Quantification kit. For the validation study, the sensitivity of the kit was tested using serial dilutions of DNA controls with different concentrations. In addition, male and female DNA reference samples were mixed in different ratios to test the detection limit of minor contributor in dilution series and to define when to proceed with autosomal STR or Y-STR analysis. Also, degraded samples were quantified to evaluate the effect of different levels of degradation in obtaining genetic profiles. The quantifications were performed on the 7500 Real-Time PCR System and the results were analyzed using the HID Real-Time PCR Analysis Software v1.2. The preliminary results revealed greater precision of Quantifiler® Trio in comparison with Quantifiler® Duo kit.

References

1. M. Barbisin et al.: Developmental validation of the Quantifiler® Duo DNA

Quantification kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. Journal of Forensic Sciences. 2009; 54: 305–319.

2. J. Liu: Direct qPCR quantification using the Quantifiler® Trio DNA quantification kit. Forensic Science International: Genetics. 2014; 13: 10 –19.
3. S. Vernarecci et al.: Quantifiler® Trio Kit and forensic samples management: A matter of degradation. Forensic Science International: Genetics. 2015; 16: 77 –85.
4. Applied Biosystems (2014). Quantifiler® Trio DNA Quantification Kit User Guide. Life Technologies.

159. QUANTIFILER TRIO DNA VALIDATION AND USEFULNESS IN CASEWORK SAMPLES

Vieira-Silva C.[1,3], Afonso Costa H.[1,3], Ribeiro T.[1,3], Porto M. J.[1,3], Dias M., Amorim A.[1,2]

[1] Instituto Nacional de Medicina Legal e Ciências Forenses, Serviço de Genética e Biologia Forenses, Portugal
[2] Faculdade de Ciências, Universidade de Lisboa, Portugal
[3] CENCIFOR – Centro de Ciências Forenses, Coimbra, Portugal

mariajoao.porto@dcinml.mj.pt

DNA profiling with sets of highly polymorfic STR markers has now been applied in various aspects of human identification. During the past few years significant progress has been made in solving technical challenges associated with STR profiling, including the ability to analyse degraded DNA, low amounts of DNA and/or the presence of PCR inhibitors. With all this constraints, DNA quantification represents an important tool to decide which method will follow in order to improve laboratory workflow. The result of these changes is that useful STR profiles can now be obtained from previously untypeable forensic DNA samples.

The new developed Quantifiler® Trio DNA method enables forensic laboratories to simultaneously obtain a quantitative and qualitative assessment of total human and human male DNA in a single, highly sensitive real-time PCR reaction. Moreover, this new method provides a quality index (QI) to detect the presence of degraded DNA along with PCR inhibitors. This guides selection of the optimal short tandem repeat (STR) analysis chemistry (autosomal, Y-STR, or miniSTR) and streamlines the workflow while increasing downstream analysis success rates.

In order to use Quantifiler® Trio DNA in our routine casework validation assays were performed concerning accuracy, sensitivity and specificity. It was also tested with a wide variety of sources, including buccal swabs, blood, semen, tissues and other human DNA sources.

The Quantifiler® Trio DNA Quantification kit proved to be a highly informative workflow tool and can function as a component of an improved and more effective processing of forensic casework samples.

160. LINEAR-AFTER-THE-EXPONENTIAL (LATE)-PCR: IMPROVED ASSSYMETRIC PCR FOR QUANTITATIVE DNA-ANALYSIS

Schulze Johann K.[1], Schürenkamp M.[1], Sibbing U.[1], Lischka C.[1], Pfeiffer H.[1], Vennemann M.[1]

[1] Institute of Legal Medicine, University Clinic Münster, Germany

Marielle.Vennemann@ukmuenster.de

Linear-after-the-exponential (LATE)-PCR describes a novel approach to assymetric PCR (1). Conventional PCR is known to plateau stochastically due to competition of binding between primers and PCR products: the more PCR products bind to the target, the less efficient the reaction becomes, because no *de novo* synthesis takes place. To avoid the plateau, assymetric PCR was describes in which massively different primer concentrations are used to transform the exponential enrichment of PCR products into a linear,

single stranded synthesis. Previous attempts to assymetric PCR showed limited PCR efficiency. To overcome this limitation, primers were designed as molecular beacons with adjusted melting temperatures, leading to increased amplification efficiencies (1).

The aim of this study was to evaluate the forensic use of LATE-PCR in quantitative analysis of both, simple and complex human STR loci.

Real time- and end-point PCR results will be presented and the potential of this technology for detecting low level and mixed DNA samples will be discussed.

1. Sanchez JA, Pierce KE, Rice JE, Wangh LJ: Linear-after-the-exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. Proc Natl Acad Sci U S A, 2004; 101(7):1933-1938

Theme 07: Predictive DNA Analysis

161. THE G-AIMS NANO SNP SET: A 31-PLEX SNAPSHOT ASSAY GENOTYPING MANY OF THE MOST INFORMATIVE FORENSIC AIMS

de la Puente M.[1], Santos C.[1], Fondevila M.[1], Manzo L.[1], the EUROFORGEN-NoE Consortium[2]; Carracedo Á.[1], Lareu M. V.[1], Phillips C.[1]

[1] *Forensic Genetics Unit, Institute of Forensic Sciences, University of Santiago de Compostela, Santiago de Compostela, Spain*
[2] www.euroforgen.eu

m.delapuente.vila@gmail.com

Massively Parallel Sequencing (MPS) allows the combination of several hundred markers in one multiplexed PCR, and this has generated a trend towards considering larger SNP sets for forensic EVC and ancestry inference. Although the forensic community is now validating MPS systems, most laboratories do not have access to the new technology nor the funds and infrastructure it requires. For this reason, we developed a SNaPshot™ assay, termed *G-AIMs Nano*, by screening the SNPs amplified in the 128-plex EUROFORGEN Global AIM-SNP set[1]. A total of 31 of the most informative markers, including three tri-allelic SNPs, were selected with the goal of maintaining a balanced differentiation of: Africans (AFR), Europeans (EUR), East Asians (EAS), Oceanians (OCE) and Native Americans (AMR). A previous forensic AIM-SNP 34-plex set designed for AFR-EUR-EAS comparisons[2], requires the genotyping of additional SNP sets when other geographic origins are involved, leading to depletion of often scant DNA extract. The *G-AIMs Nano* SNP set increases the divergence for OCE and AMR and minimizes ancestry estimation bias by keeping a five-way balance of population group differentiations. The optimized SNaPshot™ multiplex is easily implementable using standard capillary electrophoresis and allows all forensic laboratories to take advantage of the most powerful SNPs from the

Global set1. The *G-AIMs Nano* assay has successfully genotyped challenging DNA from analyses of dilution series, highly degraded material and mixed-source samples. Additionally, predictive performance for the 31 SNPs of *G-AIMs Nano* has been evaluated by analysis of samples previously characterised with much larger AIMs panels, including admixed individuals.

1. Phillips C, Parson W, Lundsberg B, et al: Building a forensic ancestry panel from the ground up: The EUROFORGEN Global AIM-SNP set. Forensic Sci Int Genet. 2014; 11:13-25.
2. Fondevila M, Phillips C, Santos C, et al: Revision of the SNPforID 34-plex forensic ancestry test: Assay enhancements, standard reference sample genotypes and extended population studies. Forensic Sci Int Genet. 2013; 7:63-74.

162. THE PREDICTION OF PIGMENTATION PHENOTYPES IN THE NORTHERN GERMAN POPULATION

Caliebe A.[1], Harder M [2], Schuett R.[2], Nebel A.[3], Krawczak M.[1], von Wurmb-Schwark N.[2]

[1] *Institute of Medical Informatics and Statistics*
[2] *Institute of Legal Medicine*
[3] *Institute of Clinical Molecular Biology*
all at Christian-Albrechts University, Kiel, Germany

caliebe@medinfo.uni-kiel.de

Human pigmentation traits are of great interest to many research areas, from ancient DNA analysis to forensic science. We developed a gene-based predictive model for pigmentation phenotypes in a realistic target population for forensic case work from Northern Germany in order to determine whether good prediction of high quality phenotypes in a genetically relatively homogeneous population is possible. We investigated the association between eye, hair and skin colour and 12 candidate single nucleotide polymorphisms (SNPs) from six genes. Our study comprised 400 individuals from Northern Germany. SNP rs12913832 in *HERC2* was found to be strongly associated with blue eye colour (OR=40.0, $p<1.2 \cdot 10^{-4}$) and to yield moderate predictive power (AUC: 77%; sensitivity: 90%, specificity: 63%, both at a 0.5 threshold for blue eye colour probability). SNP associations with hair and skin colour were weaker and genotypes less predictive.

Notably, fine phenotyping and a differentiation of hair colour into two components (light vs. dark and red tint vs. no red tint) increased the number of significant genotype-phenotype associations in the target population. A further focus of our study was the investigation of the correlation of phenotypes and the additional inclusion of phenotypes as predictors into the prediction model. Our study revealed that the prediction of one pigmentation phenotype may benefit from using information on other pigmentation phenotypes, if and when such information is available.

163. PRACTICAL EVALUATION OF SNP-BASED PREDICTION OF MALE PATTERN BALDNESS IN A SAMPLE OF CONVICTED FELONS

Eduardoff M.[1], Gandini F.[1], Berger B.[1], Pośpiech E.[2], Marcińska M.[3], The EUROFORGEN-NoE Consortium; Ballard D.[4], Phillips C.[5], Branicki W.[2,3], Parson W.[1,6]

[1] *Institute of Legal Medicine, Medical University of Innsbruck, Austria*
[2] *Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland*
[3] *Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland*
[4] *Department of Forensic and Analytical Science, Faculty of Life Science King's College, London, UK*
[5] *Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain*
[5] *Forensic Science Program, The Pennsylvania State University, PA, USA*

mayra.eduardoff@gmail.com

Androgenetic alopecia, also known as male pattern baldness (MPB), is particularly frequent among European men. Therefore, MPB represents an informative trait for forensic DNA phenotyping: the estimation of external visible characteristics that can aid the identification of perpetrators in criminal investigations. Genetic association studies have been performed in the academic arena using samples from volunteers whose MPB state was determined according to the Norwood-Hamilton scheme. In contrast, here we present an assessment of phenotype in a real-world scenario from a set of convicted felons. We investigated a previously described set of 50 candidate SNPs that have well-recorded associations with androgenetic alopecia using an in-house designed PCR-based library preparation method based on Illumina TruSeq technology. Massively parallel sequencing (MPS) of these markers allowed for further analysis of potentially associated SNPs in their sequenced flanking region. Furthermore, we reassess the phenotypic state determined by the police forces at the time of arrest according to the definitions provided in the Norwood-Hamilton scheme using arrest photographs. This data was then used to test the power of previously assessed prediction models, to enhance these models with possible novel information and to assess correlation between police determined, photo-schematic and genotypic phenotype assessments. With the growing interest in the prediction of genetically determined Externally Visible Characteristics for law enforcement purposes, analysis in a casework context will become increasingly important. This study presents a primary attempt at integrating a scientific approach in a law enforcement environment.

165. ANCESTRY PREDICTION IN SOUTH EAST ASIAN POPULATIONS USING THE ILLUMINA FORENSEQ KIT ON THE MISEQ FGX

Anantharaman R.[1], Wong Y.[1], Ng H. H.[1], Tan S. Z.[1], Syn C.[1,2]

[1] DNA Profiling Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078, Singapore
[2] Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore

ramani_anantharaman@hsa.gov.sg

Next generation sequencing in a forensic setting is fast going mainstream with the availability of the MiSeq FGx and the ForenSeq DNA Signature Prep kits from Illumina. The ForenSeq kit offers coverage of more than 200 autosomal STRs, X and Y STRs, identity, phenotypic and ancestry SNPs sequenced from 1.0ng of DNA in a single workflow. The ForenSeq Universal Analysis Software (UAS) by Illumina provides the capability to analyse the sequencing data, visualise results and perform statistical estimates of biogeographic ancestry and phenotype. The ancestry prediction capabilities in UAS are based on Principal Component Analysis (PCA) built on several reference 1000 Genomes populations.

These ancestry prediction capabilities were evaluated on samples from different South East Asian populations. Preliminary results showed that there was minimal differentiation between the tested populations as observed via the PCA in the UAS. The 1000 Genomes reference populations used by the UAS for ancestry prediction were not ideal for the segregation of South East Asian populations. To develop ancestry prediction capabilities for populations in this region, samples from each population were used as a training data set. After filtering for Hardy-Weinberg equilibrium, linkage disequilibrium and fixation index, receiver operating characteristic curves were used to identify a subset of loci with the best predictive value. Subsequently, a random, blinded selection of different samples from these populations was used as a testing data set to benchmark the ability of the selected loci to predict biogeographic ancestry. The results of this testing are presented herein.

166. ON THE USE OF THE HID-ION AMPLISEQ ANCESTRY PANEL IN A FORENSIC CASE

Hollard C.[1,2], Keyser C.[1,2,3], Vilela Lamego C.[1,3], Zvénigorosky V.[1], Delabarde T.[2,3], Ludes B.[4,5]

[1] AMIS Laboratory, CNRS UMR5288, Strasbourg, France
[2] Institute of Legal Medicine, Strasbourg University, Strasbourg, France

[3] INTS, Paris, France
[4] Institute of Legal Medicine, Paris, France
[5] Paris Descartes University, Paris, France

hollard@unistra.fr

In November 2014, a carbonized body was found in a city dump near Paris. The man did not carry any identification with him and the state of his remains prevented any direct identification. A criminal investigation opened. The autopsy did not reveal any discriminating characteristics either. Dental studies exposed extensive dental works, but failed to identify the individual, in the absence of a comparative panoramic radiograph. Genetic analyses provided a complete STR profile but it was not found in the French National Database (FNAEG). Further anthropological and radiological studies established the age at death, however they did not produce an accurate biological profile. In order for the investigation to progress, the requiring authorities allowed further genetic analyses implying markers used for biogeographical origin studies and phenotypical traits determination. In the context of this investigation, our team first used the HID-Ion AmpliSeq™ Ancestry Panel on the Ion PGM™ system (Life Technologies). The analysis of 165 AIM, improved on the biological profile but further work is needed for the results to be used as such in a criminal investigation. This work details the strategy used and difficulties met in establishing a more precise conclusion.

167. FORENSIC VALIDATION OF THE HID-ION AMPLISEQ ANCESTRY PANEL ON THE ION TORRENT PGM

Daniel R.[1], Mehta B.[2], Al-Asfi M.[2], Gahan M.[2], McNevin D.[2]

[1] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Victoria, Australia
[2] National Centre for Forensic Studies, University of Canberra, ACT, Australia

Runa.Daniel@police.vic.gov.au

Massively parallel sequencing (MPS), also known as next generation sequencing, has the potential to revolutionize forensic DNA analysis. DNA-based Forensic Intelligence relies on the analysis of DNA markers which enable prediction of biogeographical (BGA) and externally visible characteristics (EVCs) of the donor of an evidential sample. MPS enables the analysis of hundreds of DNA markers simultaneously thereby increasing the ability to provide highly detailed intelligence to forensic investigators.

The HID-Ion AmpliSeq™ Ancestry Panel (Thermo Fisher Scientific) consists of 165 autosomal SNPs selected to infer BGA[1,2]. Forensic validation criteria was applied to 96 samples using this panel to assess sensitivity (1ng-15pg), reproducibility (inter- and intra-run variability), genotype concordance and effects of compromised and forensic casework type samples (aged blood and bone, UV degraded DNA, humic acid inhibition and mixed source). Ancestry prediction accuracy was assessed using admixed (n=14) and non-admixed (n=43) samples from participants that self-declared their BGA over three generations. The samples were sequenced on two Ion 318 v2 chips (in duplicate) on an Ion Torrent PGM™ (Thermo Fisher Scientific). HID SNP Genotyper v4.3.1 software (Thermo Fisher Scientific) was used to perform ancestry predictions based on likelihood estimates.

Full SNP profiles were obtained from 0.5-1ng DNA template with a high degree of reproducibility, genotype concordance and ancestry prediction accuracy for assignment of population samples to major global populations. Assignment to sub-populations, particularly for admixed samples, requires careful interpretation as does the analysis of mixed source samples. The HID-Ion AmpliSeq™ Ancestry Panel demonstrates great potential for application to forensic DNA analysis.

1. Kosoy R *et al*. Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. Hum Mutation (2009); 30(1): 69-78
2. Kidd *et al*. Better SNPs for Better Forensics: Ancestry, Phenotype, and Family Identification. National Institute of Justice (NIJ) annual meeting, Arlington VA, June 2012 (poster).

168. POTENTIAL USE OF DIP-STR AS ANCESTRY INFORMATIVE MARKERS BASED ON THE CEPH HUMAN GENOME DIVERSITY PANEL SURVEY

Hall D.[1], Santos C.[2], Phillips C.[2], Castella V.[1]

[1] Forensic Genetics Unit, University Hospital of Lausanne, Switzerland
[2] Forensic Genetics Unit, University of Santiago de Compostela, Spain

Diana.Hall@chuv.ch

In forensic genetics, the use of ancestry informative markers to reveal the population of origin of an unidentified DNA sample can provide more information from evidentiary specimens. Although excellent results have been obtained with lineage markers, SNP and Indels, and several validated sets exist, the current resolution is mainly determined by the type of marker used. Briefly, markers with high mutation rate are informative for populations of origin, while markers with low mutation rate are ideal to assign individuals to continents.

Here, we aim at establishing the potential contribution of compound DIP-STR markers to ancestry inference, given their key feature of combining slow evolving deletion/insertion polymorphisms (DIP) to rapidly evolving STR (1).

We present the results of the first survey of 12 DIP-STR markers in a genetically interesting/reference population, that is the CEPH-HGDP panel. For most markers, the number of observed haplotypes is greater in Africa than outside Africa and it decreases with geographic distance. Interestingly, DIP-STR formed by young DIP polymorphisms originated outside Africa, show a larger number of haplotypes in Europe, Asia and America, while in Oceania the haplotype distribution is similar to Africa. These data are in agreement with the recent model of population history of the Melanesians and modern humans. Moreover, only 8 DIP-STR markers, analyzed with the algorithm STRUCTURE, can identify the groups of Africa, East Asia, Oceania, Native America and a division of Europe/Middle-East/Central-South-Asia. Further analysis of selected DIP-STR is warranted to accurately measure their contribution to ancestry inference.

1. Castella V, Gervaix J, Hall D. DIP-STR: highly sensitive markers for the analysis of unbalanced genomic mixtures. Human Mutation. (2013); 34:644-54

169. AGE PREDICTION WITH DUAL-TAQMAN PROBE SJTREC QUANTIFICATION ASSAY

Chan X. L. S.[1], Heng B.[1], Syn C. K. C[1, 2]

[1] DNA Profiling Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078, Singapore
[2] Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore

christopher_syn@hsa.gov.sg

In crime investigations, some 40% of the DNA profiles generated from the crime scene samples do not have a match in the offender DNA databases. The ability to predict externally visible characteristics (EVC) of the unknown perpetrator could facilitate police investigation by narrowing down the list of suspects. Age, while not an EVC per se, is a proxy towards the appearance of a person. Previous studies have shown that age prediction with accuracies of ±8 to ±10 years could be obtained through quantitative PCR analysis of signal joint T cell receptor excision circles (sjTRECs) using 50 ng of DNA. In the present study, the performance of a dual-Taqman® probe sjTREC quantification assay was evaluated using 222 DNA samples belonging to donors from 0 to 98 years old. Quantitation of sjTREC was normalised against the single copy gene albumin. The results indicated that positive sjTREC amplifications could be achieved with donors up to 67 years old using only 12 ng of DNA with less than 40 PCR amplification cycles. A non-linear regression model, correlating the normalised sjTREC C_T value with age, was established with a R^2 value of 0.71. From the regression model, a good prediction accuracy of ±3 years for ages up to 60 years old was obtained. Beyond 60 years of age, the regression model would be less suitable for the prediction of age as it was observed that

the number of sjTRECs appeared to plateau.

170. INTERPRETATION OF MALE PATTERN BALDNESS-ASSOCIATED DNA VARIANTS USING THE SNIPPER ONLINE CLASSIFIER

Freire-Aradas A.[1,], Pośpiech E.[2], Gómez-Tato A.[3], Álvarez Dios J.[3], Casares de Cal M.[3], Marcińska M.[2], the EUROFORGEN-NoE Consortium[4]; Branicki W.[5], Phillips C.[1], Lareu M. V.[1]

** Contributed equally to this study*
[1] Forensic Genetics Unit, Institute of Forensic Sciences, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain
[2] Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland
[3] Faculty of Mathematics, University of Santiago de Compostela
[4] www.euroforgen.eu
[5] Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland

ana.freire3@hotmail.com

Research on the genetics of male pattern baldness (MPB) is of interest for Forensic DNA Phenotyping (FDP). DNA variants associated with MPB can be used to predict scalp hair distribution in European males and thus help to extend the prediction of the visible appearance of an unknown DNA donor. Recent studies undertaken by the EUROFORGEN-NoE have developed a logistic regression model to predict MPB risk based on DNA variants in 20 SNPs from loci located on chromosomes X, 1, 2, 3, 5, 7 12, 18 and 20. The developed method showed high predictive performance in two groups of men aged less than 50 years with significant baldness (87% of correct predictions) and men aged 50 years or older lacking baldness (90% of correct predictions). The final conclusion of this study was that prediction of MPB is reasonably accurate (AUC=0.761) in European men ≥50 years old. The developed method may be relevant to forensic genetics' investigations when supported with age prediction and ancestry inference. Here we introduce a *Snipper*-based tool for interpretation of genotypes in the 20 MPB predictors identified. This is another application of the *Snipper* online classifier, which can be used to predict scalp hair distribution in males using naïve Bayes analysis.

171. COMPARISON OF DIFFERENT MATHEMATICAL METHODS FOR DNA-BASED EYE COLOUR PREDICTON APPLICABLE IN FORENSICS

Karłowska-Pik J.[1], Ziemkiewicz B.[1], Skowron M.[2], the EUROFORGEN-NoE Consortium, Branicki W.[3,4], Pośpiech E.[4]

[1] Faculty of Mathematics and Computer Science, Nicolaus Copernicus University, Toruń, Poland
[2] Department of Dermatology, Collegium Medicum of the Jagiellonian University, Kraków, Poland
[3] Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland
[4] Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland

ewelina.pospiech@uj.edu.pl

Eye colour was one of the first traits of human appearance applied with marked success in Forensic DNA Phenotyping (FDP). The developed eye-colour prediction systems mostly rely on DNA variant rs12913832 in *HERC2* and enable accurate prediction of blue and brown eye colours. Green eye colour is still difficult to predict with the currently available prediction models. In this work five mathematical approaches, including logistic regression, Classification and Regression Trees (CART), C5.0 trees, random forests and neural networks were evaluated in order to compare their effectiveness in predicting eye colour. The study involved 33 polymorphisms located in 12 genes analysed in a population of 1020 individuals from Poland. Eye colour was categorized as blue, green and brown. The developed models were tested using a 10-fold cross validation procedure, instead of the traditional testing set approach, allowing better use of the available data. The applied methods confirmed the highest significance of rs12913832 and showed similar accuracy of

prediction for blue and brown eye colours (91.2%-93.2% and 89.1%-91.2% correct predictions, respectively). Logistic regression, neural networks and C5.0 trees gave no correct predictions of green eye colour. CART trees and random forests methods gave 2.94% and 4.65% correct predictions in this category, while still maintaining a high level of blue and brown eye colour prediction (91.2%-92.3% and 89.7%-90.2%, respectively). The study showed that various mathematical methods may give slightly different prediction results using the same set of samples and predictors and this should be considered in future FDP modeling.

172. FREQUENCY DIFFERENCES OF SNP ALLELES ASSOCIATED WITH MALE PATTERN BALDNESS IN AFRICA, ASIA AND EUROPE

Marcińska M.[1], Pośpiech E.[2], Abidi S.[3], Dyrberg Andersen J.[4], van den Berge M.[5], Carracedo Á.[6,7], Eduardoff M.[8], Freire-Aradas A.[6], Morling N.[4], Sijen T.[5], Skowron M.[9], Söchtig J.[6], Syndercombe Court D.[3], Weiler N.[5], The EUROFORGEN-NoE Consortium; Ballard D.[3], Børsting C.[4], Parson W.[8,10], Phillips C.[6], Branicki W.[1,2]

[1] *Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland*
[2] *Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland*
[3] *Faculty of Life Sciences, King's College, London, United Kingdom*
[4] *Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*
[5] *Department of Human Biological Traces, Netherlands Forensic Institute, The Hague, The Netherlands*
[6] *Forensic Genetics Unit, Institute of Forensic Medicine, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain*
[7] *Genomic Medicine Group, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Institute of Health Carlos III, Spain*
[8] *Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria*
[9] *Department of Dermatology, Medical College of Jagiellonian University, Krakow, Poland*
[10] *Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania, United States of America*

magdalena.marcinska@gmail.com

Male pattern baldness (MPB) is the most common form of hair loss and is particularly frequent in European populations. Our recent study involving 605 males from 7 European populations confirmed that the *AR/EDA2R* region on the X-chromosome and a locus on 20p11 are two major genetic determinants of MPB in Europe. Little is known about DNA variants associated with hair loss in African and Asian males but the available data show considerably lower incidence of MPB in these populations. Here we used the 1000 Genomes data to compare frequencies of MPB-associated alleles previously identified as affecting European males, in populations from Africa, Europe, South Asia and East Asia. In European populations, MPB-associated alleles were found to be highly prevalent with frequencies >50% for all SNPs located on 20p11 and >70% for AR/EDA2R SNPs. Substantially lower frequencies were noted for most European MPB alleles on 20p11 in Asia and Africa. However, allele G in rs1998076 that was most associated with MPB in our study (frequency: 61%, OR: 1.85, *p:* 2.941×10^{-4}), was found to be particularly frequent in Africa (78%). Moreover, the whole set of MPB-associated alleles on the X-chromosome was found to be monomorphic in Asians. In Africans, the X-chromosome risk allele C in rs1397631 was very frequent (84%) and in a single LD block with rs5919324; the marker most strongly correlated with MPB in our study (OR: 1.87, *p:* 1.119×10^{-4}). This investigation emphasizes the complex genetic architecture of hair loss in men and stresses the need for further studies based on more extensive population surveys from wide ranging geographic regions.

173. ASSESSING THE SUITABILITY OF DIFFERENT SETS OF INDELS IN ANCESTRY ESTIMATION

Aquino J.[1], Jannuzzi J.[1], Carvalho E. F.[1], Gusmão L.[1,2,3]

[1] *DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil*

[2] IPATIMUP (Institute of Pathology and Molecular Immunology from the University of Porto), Porto, Portugal
[3] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

lgusmao@ipatimup.pt

Ancestry Informative Markers (AIMs) are useful to estimate individual and population ancestries, providing important information to forensic investigations. Several AIM sets were described and evaluated by comparison with data from GWAS. Taking into account that an efficient set of AIMs shall provide identical results between full brothers and GWAS are not easily performed, we aimed to see if the accuracy of the ancestry estimates are correlated to differences obtained in siblings. Pairs of siblings from Brazil were genotyped for 83 INDELs; and values of African, European and Native American contributions were calculated using the 30 markers with lowest (set 1) and the highest (set 2) inter-population variation. Despite the low efficiency of set 1 to estimate ancestry, it presented lower differences between siblings than set 2. Values of ancestry below 0.33 for set 2 were always overestimated by set 1, and higher values were underestimated. The non-random deviation of estimates for set 1 precludes the usefulness of a comparative analysis in siblings. Results were also compared for set 2, 46 AIMs and the 83 full set. The differences observed between pairs of sets were apparently random, which makes the comparison of the ancestry in siblings meaningful. Although not significantly different, the highest average differences between brothers were found for the 30 markers' set, followed by the 46 AIMs and were lower for the full set of 83 markers, supporting a better performance of the complete set of markers in ancestry estimation, although including markers with low inter-populations variation.

174. TYPING OF 111 ANCESTRY INFORMATIVE MARKERS IN AN ALBANIAN POPULATION

Ribeiro J.[1], Pereira V.[1], Kondili A.[2], Miniati P.[2], Børsting C.[1], Morling N.[1], EUROFORGEN-NoE

[1] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[2] Scientific Support Section, Subdivision of Biological and Biochemical Examinations and Analyses, F.S.D. – Hellenic Police, Athens, Greece

vania.pereira@sund.ku.dk

Genetically based prediction of bio-geographic ancestry has a great potential in forensic genetics and may be used as an investigative lead in crime case work or disaster victim identifications.

The Euroforgen-NoE consortium developed four PCR and SBE multiplexes for typing of 111 ancestry informative markers (AIMs) with the purpose of differentiating Middle Eastern populations from the rest of the world (publication in preparation). Before these multiplexes can be applied in forensic case work, population data for these markers are needed.

In this work, a collection of samples from Albania was genotyped and, at the same time, the performance of each SNP assay was evaluated. After PCR and SBE, samples were genotyped using the Sequenom MassARRAY. All samples were typed at least two times. The mass spectra were analysed using Typer 4 and the genotype calls were further analysed with a custom designed script in the software R.

The results were compared to other population samples previously typed for the same markers. A multidimensional scaling analysis was carried out using pairwise genetic distance information.

175. STUDIES OF EAST EUROPEAN POPULATIONS WITH A 46-PLEX ANCESTRY-INFORMATIVE INDEL SET

Bulbul O.[1], Duvenci A.[1], Zorlu T.[1], Gurkan C.[2], Santos C.[3], Phillips C.[3], Lareu M. V.[3], Filoglu G.[1]

[1] Institute of Forensic Science, Istanbul University, Turkey
[2] Turkish Cypriot DNA Laboratory, Nicosia, North Cyprus

[3] Forensic Genetics Unit, Institute of Forensic Sciences, University of Santiago de Compostela, Spain

oslemblbl@yahoo.com

Insertion-deletion polymorphisms (InDels) have many characteristics (including low mutation rates, short amplicon sizes and a very straightforward amplification technique) that make them well suited to forensic DNA analysis. InDels are also practical loci for the forensic analysis of biogeographic ancestry as they can show high allele frequency differentiation amongst population groups, while mixed DNA is less likely to go undetected and be misinterpreted as indicating admixed ancestry. In this study we studied patterns of genetic variation from 46 ancestry-informative InDels in Turkish, Turkish Cypriots and Azerbaijani populations. This study provided new data for a forensic InDel database based on the SPSmart frequency browser framework: *forInDel (Forensic Indel browser)*. 40 unrelated individuals were genotyped from each population and ancestry estimates were then assessed using HGDP-CEPH samples as reference data. No departures from Hardy-Weinberg equilibrium were detected. Ancestry analyses used Structure, Fst comparisons and principal component analysis. Results indicated the 46 InDel set, though highly efficient in inferring the ancestry of individuals from Africa, Europe and East Asia, did not reveal distinct genetic clusters for Middle Eastern and Central South Asian populations. Populations from Turkey, Cyprus and Azerbaijan all show high European ancestry components. Lastly, it is likely that Eurasian sub-population groups will be more optimally differentiated by combining the InDels used with extended sets of InDels focused on variation within Eurasia as well as with the addition of panels of ancestry-informative SNPs chosen for the same purpose.

176. INFERRING SOUTHWEST ASIAN ANCESTRY WITH AN EFFICIENT PANEL OF SNPS

Bulbul O.[1,2], Cherni L.[3], Filoglu G.[2], Kidd K. K.[1]

[1] Department of Genetics, Yale University School of Medicine, New Haven, CT, USA

[2] Institute of Forensic Science, Istanbul University, Turkey

[3] Laboratory of Molecular Genetics, Immunology and Human Pathology, Faculty of Sciences of Tunis, University El Manar, Tunisia

ozlembulbul00@gmail.com

Many different published sets of single nucleotide polymorphisms (SNPs), insertion-deletion polymorphisms (InDels), etc. can serve as ancestry informative markers (AIMs) to distinguish among continental regions of the world. The Kidd Lab panel of 55 ancestry-informative SNPs (AISNPs) provides good global reference data (cf. FROG-kb <frog.med.yale.edu>) and distinguishes at least 8 biogeographic ancestry groups. We aim to identify the best subset of those 55 SNPs for inferring ancestry among Southwest Asian populations as an initial step toward a small, efficient, low cost forensic panel focused on this region of the world. We used 3129 individual from 52 populations for analyses focused on populations from the area: East Africa, North Africa, Europe, South West Asia, and South Asia. Using heatmap and Fst analyses we eliminated essentially redundant SNPs and those providing no information on populations within this region (i.e., varied only in other parts of the world). In STRUCTURE and PCA analyses, the remaining selected 32 SNPs out of the 55 provide the same clear differentiation of five population groups (Europe, Mediterranean, Middle East, Central South Asia and Africa) as the full 55 AISNPs. Our next step toward an optimized panel for this region will be to highly informative SNPs selected from other sources, type them on these 3129 individuals, and add them to the dataset. Reiterating the refinement procedures with all new markers added should result in an improved panel of AISNPs that provides additional information on differences among populations in this part of the world.

177. AN ALTERNATIVE METHOD FOR THE EVALUATION OF THE ETHNIC ORIGIN OF UNKNOWN GENETIC PROFILES

Alladio E.[1,2], Vincenti M.[1,2], Testi R.[2,4], Garofano P.[2,3]

[1] Dipartimento di Chimica, Università degli Studi di Torino, Torino, Italy
[2] Centro Regionale Antidoping e di Tossicologia "A. Bertinaria", Orbassano (TO), Italy
[3] Laboratorio Biologia Forense - Centro Regionale Antidoping "A. Bertinaria", Orbassano (TO), Italy
[4] ASL TO2 Regione Piemonte, SC Medicina Legale, Torino, Italia

ealladio@unito.it

DNA typing and Short Tandem Repeats (STRs) data interpretation are one of the most investigated fields in forensic science and the evaluation of STRs' capabilities for distinguishing ethnic groups/populations has been largely examined[1,2]. In order to increase crime investigation elements when unknown subjects are involved, it would be extremely useful to determine the ethnic origin from DNA profiles. Until now, genetic profile methods for ethnic origin estimation is based on population alleles' frequencies that quantifies the evidence according to bayesian reasoning in terms of likelihood ratio, supporting the hypothesis that a certain profile belongs to one rather than another ethnic group. In particular, this work provides an alternative approach to the likelihood ratio method for the estimation of the African-American, Asian, Caucasian or Hispanic origins of unknown biological traces. Chemometrics application could represent a powerful tool for investigating authorities when biological evidence are collected at crime scene, during mass disaster or missing person investigations. Powerful multivariate techniques such as Principal Component Analysis (PCA), Partial Least Squares-Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM) were successful tested. In particular, they were experimented on NIST U.S. population database [3], which contain allele frequencies of 29 autosomal STR loci relevant to U.S. African-American, Asian, Caucasian and Hispanic populations. More in details, PLSDA and SVM techniques provided robust classifications. Models with high sensitivity and specificity for different population discrimination were developed. Future perspectives are relevant for the application of these chemometric approaches to discriminate more locally-restricted populations.

1. Brenner CH: Difficulties in estimating ethnic affiliation (letter). Am. J. Hum. Genet. 1998; 62: 1558 – 1560.
2. Brenner CH: Probable Race of a Stain Donor, Proceedings from the Seventh Human Identification Symposium. 1996; 48 – 52.
3. Hill CR, Duewer DL, Kline MC, Coble MD and Butler JM: U.S. population data for 29 autosomal STR loci. Forensic Sci. Int. Genet. 2013; 7: e82 – e83.

178. POPULATION STUDIES ANALYSING VARIATION IN A 46-PLEX AIM-INDEL FORENSIC ANCESTRY ASSAY

Santos C., Oldoni F., Fondevila M., Phillips C., Lareu M. V.

Forensic Genetics Unit, Institute of Forensic Sciences, University of Santiago de Compostela, Spain

c.phillips@mac.com

Several ancestry-informative marker (AIM) sets have been developed for forensic analysis of ancestry. A key factor in the use of AIMs is the availability of well-characterized reference population data to ensure more precise prediction of ancestry, especially when unknown individuals have complex admixture patterns. In this study we have expanded the population coverage of a set of 46 AIM-Indels [1] beyond the standard starting point of the HGDP-CEPH diversity panel. These extended population studies also allowed the identification of rare and sometimes group-specific novel insertion-deletion alleles amongst the 46 markers. We genotyped and estimated allele frequencies for 353 samples from 10 populations: Ghana (Gh), Sierra Leone (SL), Nigeria (Ng), Zimbabwe (Zb), Somalia (Sm), Galicia (Gl), Morocco (Mr), Turkey (Tk), Venezuela (Vz) and Guatemala (Gt). The efficiency of the AIM-Indels assay in making an ancestry inference and estimating ancestry proportions at the individual level was evaluated with reference population data from the HGDP-CEPH panel using *STRUCTURE*, PCA and *Snipper* as independent but complimentary methods of analysis. Results indicated seven of the study populations cluster with the expected major ancestry group: Gh, SL, Ng, Zb with African; Gl mainly

European; and Vz, Gt mainly Native American. As previously described [2], the Tk sample shared ancestry between European and Central South Asian reference data. Lastly, admixture patterns were detected in Sm (African/European admixed) and Mr that showed similar patterns to CEPH Algerian Mozabites (majority European ancestry with a minor African component). All population data is available in the online *forInDel* frequency browser [3].

References

[1] Pereira R, Phillips C, Pinto N et al.: Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. PLoS One (2012); (7): e29684.

[2] Phillips C, Freire-Aradas A, Kriegel AK et al.: Eurasiaplex: a forensic SNP assay for differentiating European and South Asian ancestries. Forensic Sci. Int. Genet. (2013); (7): 359-366.

[3] http://spsmart.cesga.es/forindel.php

179. USE OF ANCESTRY-INFORMATIVE MARKERS AS A SCIENTIFIC TOOL TO COMBAT THE ILLEGAL TRAFFIC IN HUMAN KIDNEYS

Severini S.[1], Carnevali E.[1], Margiotta G.[1], Garcia-González M. A.[2], Carracedo Á.[3]

[1] *Institute of Legal Medicine, University of Perugia, Italy*
[2] *Clinical University Hospital (CHUS) of Santiago de Compostela, Spain*
[3] *Institute of Legal Medicine, University of Santiago de Compostela, Spain*

simonaseverini@gmail.com

In the process of development of modern medicine, organ transplants represent one of the most important and more meaningful achievements, allowing to extend and improve the lives of patients around the world. However, increasing requests and the global lack of organs have created the basis for a highly profitable black market in organs that promotes criminal enterprise.

This study was focused on illegal kidney traffic and our aim was to develop a reproducible and cheap method based on urine sediment that could be used in cases of transplanted organs for which there is poor and vague information about the donor.

The basic considerations that supported this work were two: 1) the populations involved in the trafficking of kidneys are well characterized and genetically distinct: 2) urine of a transplant recipient contains DNA from both the donor and the recipient: the genetic profile of the recipient can be obtained analyzing his oral swab while the donor's one can be obtained simply by subtraction.

Eighty urinary samples from subjects who have undergone kidney transplant has been analyzed with the 34plex SNP forensic ancestry test (developed at University of Santiago de Compostela, Spain) [1-2] that can predict the geographical origin of a subject discriminating between the three major population groups: Europe, sub-Saharian Africa and East Asia. In many samples comparison between the profile obtained analyzing the DNA extracted from the urine and the one obtained from the oral swab of the same subject, allowed to determine the geographical origin of the donor.

1. Phillips C, Salas A, Sanchez JJ, Fondevila M, Gomez-Tato A, Alvarez-Dios J, Calaza M, Casares de Cal M, Ballard D, Lareu MV, Carracedo Á, The SNPforID consortium: Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs. Forensic Science International: Genetics. 2007; 1: 273–280
2. Fondevila M, Phillips C, Santos C, Freire Aradas A, Vallone P.M, Butler JM, Lareu MV, Carracedo Á: Revision of the SNPforID34-plex forensic ancestry test: Assay enhancements, standard reference sample genotypes and extended population studies. Forensic Science International: Genetics. 2013; 7: 63-74

180. CORRELATION BETWEEN THE MOLECULAR BASIS OF IMPULSIVITY AND SUICIDAL BEHAVIOR

Barrot C.[a], Ortega M.[a] Carrera C.[a], De Alcaraz-Fossoul J.[a], Subirana M.[b], Salavert J.[c], Castellà J.[d], Mezquita J.[e], Gené M.[f]

[a] *Legal Medicine and Toxicology Unit. Public Health Department. University of Barcelona. Catalonia*
[b] *Cap de Secció d'Anatomia Forense IMLC. Catalonia*
[c] *Dept. Psiquiatria. Hospital de Sant Rafael. Barcelona. Catalonia*
[d] *Cap de Servei de Patologia Forense IMLC. Catalonia*
[e] *Dept Ciències Fisiològiques I. University of Barcelona. Catalonia*
[f] *Dept Ciències Clíniques. University of Barcelona. Catalonia*

Suicidal behavior is, in most cases, a complication of a psychiatric disorder. Over 90% of people who die as a result of suicide have a psychiatric illness. A recent analysis of data from the National Comorbidity Survey indicated that depression was the factor that was most often associated with the onset of suicidal thoughts. However, other disorders related to the control of impulses, anxiety and agitation showed to play a stronger role in the progression from a suicidal thought to the actual suicide attempt.

In the etiology of suicide, besides psychopathological factors, other influencing causes have to be considered, including neurobiological, genetic, social, and religious aspects. The correlation among impulsivity, aggressiveness and suicide has led to the association of the psychopathological basis of suicidal behavior with stressful situations and alterations in neurotransmitters(Apter et al., 1990). The latter suggests that certain gene polymorphisms must be either directly involved in these genetic pathways or implicated in their expression. Our studies focus on evaluating the relationships between polymorphisms and the serotonin receptor 1B (HTR1B), which has been related with the aggressive behavior of laboratory animals (Bach & Arango, 2012). Another enzyme being assessed is TPH1 (Courtet et al., 2004), which is associated with impulsivity.

1. Apter, A., van Praag, H. M., Plutchik, R., Sevy, S., Korn, M., & Brown, S.-L.. Interrelationships among anxiety, aggression, impulsivity, and mood: A serotonergically linked cluster? Psychiatry Research, 1990, 32(2), 191–199.
2. Bach, H., & Arango, V.. Neuroanatomy of Serotonergic Abnormalities in Suicide. In Dwivedi Y (Ed.), The neurobiological basis of suicide. Boca Raton (FL): CRC Press; 2012, 11–28
3. Courtet, P., Jollant, F., Castelnau, D., Astruc, B., Buresi, C., & Malafosse, A.. Implication of genes in the serotonergic system for vulnerability to suicidal behaviors. Journal of Psychiatry and Neuroscience, 2004, 29(5), 350–359.

181. INVADER ASSAYS FOR RAPID ANALYSIS OF PHENOTYPIC TRAITS

Clarisse L.[1], Imagawa R.[3], Sato H.[4], Kawahara M.[4], Kubo M.[2], Sijen T.[1], Westen A.[1]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*
[2] *The RIKEN Center for Integrative Medical Sciences, Yokohama, Japan*
[3] *Toppan Printing Co. Ltd., Tokyo, Japan*
[4] *Riken Genesis Co. Ltd., Tokyo, Japan*

t.sijen@nfi.minvenj.nl

Since 2010, the NFI offers a rapid STR-typing service to the Dutch police. Rapid profiling is achieved by amplifying a limited amount of evidentiary material collected via mini tape lifts in a direct PCR. Subsequently, the STR profile is searched against the DNA database and in case of a match, a name is given as an investigative lead to the police within a few hours. When no match is obtained, it would be useful to rapidly extract other intelligence information from the trace, such as phenotypic traits. Here, we describe an assay that generates genotyping data to predict gender, eye, hair and skin color within 2 hours. Like with rapid STR typing, mini tape lifts are used to collect a limited amount of evidentiary material. These tape lifts are subjected to a 10 min DNA purification protocol by which a low volume DNA extract is obtained. The markers for gender and 30 eye, hair and skin color SNPs are co-amplified in

a PCR of 40 amplification minutes. Single-plex Invader assays are performed taking 25 min on standard real time PCR equipment. To reduce the handling time, chemicals of the Invader assay are pre-dried in 96-wells plates. Validation experiments were performed, resulting in full profiles with 63 pg or more DNA. As the assay cannot be overloaded, inputs of 5 ng DNA also gave complete genotyping data. Excellent success rates were obtained for simulated forensic casework samples containing blood.

Theme 08: DNA Typing Methods

182. GENETIC MARKERS FOR SEX IDENTIFICATION OF THAIS IN FORENSIC DNA ANALYSIS

Sanubol A.[1], Thooptianrat T.[1], Yimpongsawat N.[1], Faijaidee W.[1], Chaveerach A.[1,2], Sudmoon R.[1,2], Tanee T.[2,3], Yaipool K.[4]

[1] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand
[2] Genetics and Environmental Toxicology Research Group, Khon Kaen University, Khon Kaen, Thailand
[3] Faculty of Environment and Resource Studies, Mahasarakham University, Mahasarakham, Thailand
[4] Faculty of Law, Khon Kaen University, Khon Kaen, Thailand

raccha@kku.ac.th

The sex chromosome marker is currently in widespread use for identification of chromosomal sex of an unknown DNA sample and differentiating the relative individuals of male and female DNA in a mix forensic sample. So, the research aimed at conduction of sex determination using a standard primer-specific set of three primer regions namely amelogenin and two centromeric alphoid repeats on Thais. The hair shafts with roots of 73 female and 72 male individuals collected from southern, northern, central and northeastern Thailand were DNA extracted and tested with the primers specific-sex regions mentioned by DNA amplification. The results showed that the studying on amelogenin and centromeric alphoid repeat 2 regions have error 10.96 and 5.56% with absence or missed banding sizes, success 89.04 and 94.44%. The most effective region as benefit 100% for Thais specific sex identification is the centromeric alphoid repeat 1. The region is suitably for sex chromosome marker on Thais in forensic DNA analysis.

183. A NOVEL SYSTEM FOR FORENSIC SNP ANALYSIS THROUGH PCR-LIGASE DETECTION REACTION

He W.[1], Mao J.[2], Bai X.[2], Feng T.[2], Wang L.[1], Li Z.[1], Zu W.[1], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China

liangweibo@scu.edu.cn

With the advantages of lower mutation rates, and improved analysis of the degraded or aged samples, single nucleotide polymorphism (SNP) markers has been considered as supplementary to the STRs. Consequently, a robust, simple and cost effective technique for SNP typing is essential to analyze a large number of SNPs. Ligase Detection Reaction (LDR) could detect a SNP on CE platform through the ability of DNA ligase which can seal the nick junction formed by oligonucleotides hybridized to the flank region of target SNP. Here we purposed a prestudy to set up a 6 Y-SNPs multiplex panel for forensic application through PCR-Ligase Detection Reaction (LDR) system. Primers of 6 loci were designed for multiplexed PCR. All the amplicons were less than 100 bps for easy detection of degraded or aged DNA samples. According to the sequence around the SNP site, three LDR probes were designed for each SNP including one common fluorescent labeled probe and two allele specific probes different in size with mismatching bases at the 3′ end. PCR-LDR products were profiled through ABI3130 Genetic Analyzer. A set of 6 Y-SNP could be profiled through one multiplexed PCR and one multiplexed LDR. Even though the number of markers in the current system is limited, when fully developed, it can easily be multiplied more SNPs and yield a greater power of discrimination. Our study indicated that the PCR-LDR could provide a robust, simple and cost effective multiplexed SNP typing method for forensic cases in the future.

184. IMPROVING SAMPLE ANALYSIS AND INTERPRETATION USING QIAGENS LATEST INVESTIGATOR STR MULTIPLEX PCR ASSAYS WITH A NOVEL QUALITY SENSOR

Scherer M., König M., Breitbach M., Cornelius S., Müller D., Bussmann M., Prochnow A., Peist R.

QIAGEN GmbH
Qiagenstrasse 1 40724 Hilden Germany

Mario.scherer@qiagen.com

The European STR standard set (ESS) of loci as well as the new expanded CODIS core loci set as recommended by the CODIS Core Loci Working Group, has led to a higher standardization and harmonization in STR analysis across borders. Various multiplex PCRs assays have since been developed for the analysis of these 17 ESS or 23 CODIS expansion STR markers that all meet high technical demands.

However, forensic analysts are often faced with difficult STR results and the questions thereupon. What is the reason that no peaks are visible in the electropherogram? Did the PCR fail? Was the DNA concentration too low? QIAGEN's newest Investigator STR kits contain a novel Quality Sensor (QS) that acts as internal performance control and gives useful information for evaluating the amplification efficiency of the PCR. QS indicates if the reaction has worked in general and furthermore allows discriminating between the presence of inhibitors or DNA degradation as a cause for the typical ski slope effect observed in STR profiles of such challenging samples. This information can be used to choose the most appropriate rework strategy.

We show data of the Investigator ESSplex SE QS Kit addressing the ESS and the Investigator 24plex QS Kit addressing the CODIS expansion set, both with integrated quality sensor.

185. EARLY NON-INVASIVE PRENATAL PATERNITY TEST BASED ON DIP-STR ANALYSIS

Hall D.[1], Irion O.[2], Castella V.[1]

[1] Forensic Genetics Unit, University Hospital of Lausanne, Switzerland
[2] Obstetrics and Gynecology, University Hospital of Geneva, Switzerland

Diana.Hall@chuv.ch

An early non-invasive prenatal paternity test within a forensic framework could help all those women who get pregnant during the time of a sexual abuse. This is possible with the analysis of the small quantities of DNA of the fetus that circulate in the maternal blood (1-5% of total DNA starting at 6 weeks of pregnancy) and by using genetic markers capable of distinguishing the fetal DNA from the maternal background. Unfortunately, the use of standard Y-STRs only applies to baby-boys and the results identify a male linage.

In this study, we aim at establishing whether the novel compound genetic markers DIP-STR (1), developed for the analysis of unbalanced DNA mixtures, can be used to determine paternity in pregnancy. DIP-STRs have the advantage of being genome-wide located, and therefore they can target the fetal DNA of any sex, even when this contributes less than 0.1% of DNA.

Plasma DNA from 50 pregnant women was analyzed with a panel of 10 DIP-STR, longitudinally (1st, 2nd and 3rd trimester of pregnancy). The initial analysis of 34 families showed 26 cases with 3 to 6 informative markers (markers showing one or two paternal alleles that can be targeted by PCR) and only 6 cases with one informative marker. Overall, the paternally transmitted DNA was successfully detected at all time points. However, the samples collected during the first trimester sometimes required a larger volume of plasma. Further research in statistics is ongoing to establish the appropriate framework for this novel type of paternity test.

1. Castella V, Gervaix J, Hall D. DIP-STR: highly sensitive markers for the analysis of unbalanced genomic mixtures. Human Mutation. (2013); 34:644-54

186. AN EVALUATION OF MINISTR MARKERS IN CASEWORK SAMPLES

Alghafri R.[1], Almheiri R.[1]

[1] Department of Forensic Sciences and Criminology, Dubai Police G.H.Q., Dubai, United Arab Emirates

r.alghafri@hotmail.co.uk

The analysis of mini short tandem repeats (miniSTRs) is a useful tool in various contexts of forensic DNA analysis. Main application is the identification of DNA profiles from degraded DNA samples. In the present study, previously identified autosomal miniSTR markers were incorporated in single multiplex assay in order to evaluate the use of such markers in forensic casework samples [1]. Seven autosomal miniSTR markers comprise of D12ATA63, D17S974, D9S2157, D2S1776, D10S1435, D1S1627 and D3S4529 were chosen based on previous performance study [1]. Sensitivity of the assay was tested down to 31.25 pg of DNA. Allele's frequencies were established using 735 randomly collected samples from United Arab Emirates Arab population. Forensic parameters including matching probability, power of discrimination, paternity power of exclusion and typical paternity index were calculated using Arlequin v3.5 software. Usefulness of these markers were evaluated in 100 forensic casework samples which have shown partial or non profiles using commercially available autosomal multiplex assay (Identifiler® Plus STR amplification Kit).

1. Carolyn R. Hill M.S., John M. Butler Ph.D. and Peter M. Vallone Ph.D. A 26plex Autosomal STR Assay to Aid Human Identity Testing. Journal of Forensic Sciences. 2009; 54:page 1008 – page 1015.

187. AN EVALUATION OF A NEW RAPID DNA PLATFORM FOR FIELD FORWARD APPLICATIONS

LaRue B. L., Budowle B.

Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA

Bobby.Larue@unthsc.edu

Generation and upload of DNA profiles outside the traditional laboratory setting can be instrumental to improve workflow and reduce backlogs for DNA typing. The RapidHIT ID™ (IntegenX; Pleasanton, CA, USA) system is a second generation Rapid DNA system that is configured to perform DNA extraction, PCR amplification electrophoresis, and data analysis of reference swabs with an expert system to generate forensic DNA profiles comparable to traditional bench systems. The RapidHIT ID system has a novel design that reduces its footprint and number of samples that must be run at any one time. Reliable STR profiles from reference buccal swabs were obtained with nominal "hands-on" sample loading time and with a signficant enhancement of workflow compared to the first generation Rapid DNA systems. The RapidHIT ID system was tested for reliability and concordance, and interpretation thresholds were determined. The new instrument provided results comparable with those from traditional DNA genotyping methodologies.

188. DIRECT-TO-PCR TISSUE PRESERVATION FOR DNA PROFILING

Sorensen A.[1], Berry C.[2], Bruce D.[3], Gahan M.[2], Hughes-Stamm S.[1], McNevin D.[2]

[1] *Department of Forensic Science, Sam Houston State University, Texas, USA*
[2] National Centre for Forensic Studies, University of Canberra, Australia
[3] NSW Forensic and Analytical Science Service, Sydney, Australia

dennis.mcnevin@canberra.edu.au

Tissue preservation offers the ability to stabilise and isolate DNA from tissues in the field, far from a laboratory setting, where refrigeration may not be available. This has potential application to disaster victim identification (DVI) as well as to any form of field based forensic biological evidence or intelligence collection[1]. Forensic DNA analysis is one of the three primary methods of identification recommended by the International Criminal Police Organisation (INTERPOL), together with fingerprint and dental analysis[2]. In previous work, we have demonstrated the ability to obtain full AmpFℓSTR® Identifiler® (Life Technologies) STR profiles from DNA extracted from fresh muscle tissue preserved in TENT buffer (Tris, EDTA, NaCl, Tween 20), salt-saturated DMSO-EDTA solution (DESS) and two proprietary preservatives: DNAgard® (Biomatrica) and one from DNA Genotek, Inc[3]. Three of the preservatives (DESS, DNAgard and DNA Genotek) also yielded full profiles from DNA extracted from aliquots of the preservative solution surrounding the muscle tissues. In this study, we explored the possibility of obtaining DNA profiles without DNA extraction, by adding aliquots of preservative solutions surrounding fresh and decomposing human tissue samples directly to PCR. We obtained full PowerPlex® 21 (Promega) and GlobalFiler® (Life Technologies) DNA profiles from fresh and decomposed tissue preserved at 35 °C for up to 28 days as well as from fresh tissue which had been stored at 35 °C for up to 28 days, and then at -80 °C for four years.

1. Montelius K, and Lindblom, B: DNA analysis in disaster victim identification. Forensic Science, Medicine, and Pathology. 2012; 8(2): 140-147.
2. INTERPOL: Disaster Victim Identification Guide. 2009; Lyon.
3. Allen-Hall A and McNevin D: Human tissue preservation for disaster victim identification (DVI) in tropical climates. Forensic Science International: Genetics. 2012; 6(5): 653-657.

189. A REVIEW OF SINGLE SOURCE LIVE CASE WORK SAMPLES WITH OPTIMAL DNA INPUTS PROCESSED WITH THE POWERPLEX ESI17 FAST KIT

Moore D.[1], Thomson J.[1]

[1] *LGC, Teddington, United Kingdom*

David.moore@lgcgroup.com

LGC validated the PowerPlex® ESI17 Fast system (Promega) in July 2014. As part of the validation a characterisation study was carried out to assess kit performance at different DNA inputs. This study provided information about expected peak heights, heterozygous balance and stutter proportions for the different input amounts of DNA.

Following implementation of the PowerPlex® ESI17 Fast system, reports of lower than expected peak heights and heterozygous imbalance being present in profiles from optimal input (500pg) samples were made. A review of profile quality for single source samples with optimal DNA input was carried out. A number of variables were assessed for association with the observed reduced peak height and heterozygous imbalance events. Sample degradation was observed to be the major cause of reduced peak heights and heterozygous imbalance in samples with optimal DNA input.

Evidence of DNA degradation was observed in ~50% of samples reviewed. Although the PowerPlex® ESI17 Fast system provides smaller amplicons for the loci that are required to load to the UK National DNA database; the larger number of loci present within the kit results in a larger number of large amplicons that are more likely to be affected by DNA degradation.

191. DEVELOPMENT OF A SNP-STRS MULTIPLEX FOR FORENSIC IDENTIFICATION

Wang L.[1], He W.[1], Mao J.[2], Wang H.[2], Jin B.[1,3], Liang W.[1], Luo H.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China
[3] Department of Forensic Medicine, North Sichuan Medical College, Nanchong 637000, PR China

liangweibo@scu.edu.cn

The short tandem repeat (STR) and single nucleotide polymorphisms (SNP) widely exist in human genomes. A SNP-STR, a compound genetic marker combining a STR locus with tightly linked SNPs, is more informative than any single polymorphism. The method of amplification refractory mutation system (ARMS) can allow DNA sample with specific base (e.g. SNP) amplify successfully, which avoids the common PCR bias in the analyses of mixtures. The aims of this study are enriching the rs25768-D5S818 (D5S818, from U.S. Core Loci) primers got in the previous research to develop a forensic SNP-STRs multiplex. SNPs rs4847015 and rs2246512 in the 5′ flanking regions of STRs D1S1656 and D10S1248 (from Extended European Standard Set) respectively were screened from the UCSC genome browser. Two forward SNP allele-specific primers labeled with different fluorescent dyes (Joe for A, 6-FAM for G) for each SNP-STR maker were designed with the ARMS, and a common reverse primer is located at the 3′ region of STR. The amplicons of the three SNP-STRs multiplex were profiled via Genetic Analyzer ABI 3130, and all the peaks were balanced. The high sensitivity and specificity of the multiplex were confirmed by using the standard DNA (9947A and 9948). The SNP-STR genotype of minor component (0.05ng) in the artificial extremely unbalance two DNA mixture (ratio 1:40) was detected. For the further applications of forensic genetic practice cases, more SNP-STRs should be selected to enlarge the multiplex in the following researches.

192. A STRATEGY FOR THE CHARACTERIZATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN A REFERENCE MATERIAL

Kiesler K. M.[1], Gettings K. B.[1], Vallone P. M.[1]

[1] National Institute of Standards and Technology, Gaithersburg, MD, USA

Kevin.Kiesler@NIST.gov

The advent and adoption of next generation sequencing (NGS) is enabling analysis of single nucleotide polymorphisms (SNPs) at an unprecedented scale, limited primarily by multiplexing during the PCR amplification based enrichment step used for forensic applications. Since only a single nucleotide

is assayed, PCR primers may be designed to generate small amplicons, making SNP markers well-suited to forensic typing. Carefully selected panels of SNP markers have been previously established for forensic applications such as estimating biogeographical ancestry, phenotype predictions, as well as one to one matching [1,2,3,4]. To support the implementation of SNPs in forensic DNA analysis, NIST has examined the Ion Ampliseq Identity Panel and the Ion Ampliseq Ancestry Panel for the Ion Torrent Personal Genome Machine (PGM) and the Illumina ForenSeq DNA Signature kit for the Illumina MiSeq FGx. In total, over 300 SNP markers were typed with approximately 80 % of loci being represented in more than one multiplex. A strategy combining NGS and Sanger sequencing for characterizing the markers for varying levels of confidence will be discussed. The goal is to report only the SNP allele calls, analogous to the short tandem repeat markers in SRM 2391c, and not the subsequent application of the information (e.g. actual prediction of ancestry or phenotype). Additionally, we demonstrate the optimization of automated sequencing template preparation using the Ion Chef from Ion Torrent to create an efficient workflow suitable to the forensic DNA laboratory.

1. Nassir R, Kosoy R, Tian C, White PA, Butler LM, Silva G, Kittles R, Alarcon-Riquelme ME, Gregersen PK, Belmont JW, De La Vega FM and Seldin MF: An ancestry informative marker set for determining continental origin: validation and extension using human genome diversity panels. BMC Genetics. 2009; 10:39
2. Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, Branicki W and Kayser M: The HIrisPlex system for simultaneous prediction of hair and eye colour from DNA. Forensic Science International: Genetics. 2013; 7: page 98 – page 115
3. Musgrave-Brown E., Ballard D., Balogh, K. et al.: Forensic validation of the SNP*for*ID 52-plex assay. Forensic Science International: Genetics. 2007; 1: page 186 – page 190
4. Pakstis AJ, Speed WC, Kidd JR and Kidd KK: Candidate SNPs for a universal individual identification panel. Human Genetics. 2007; 121: page 305 – page 317

193. NON-BINARY SNPS REVISITED: A NEW FORENSIC SNAPSHOT ASSAY OF 29 MULTIPLE-ALLELE SNPS

Manzo L.[1,2], de la Puente M.[1], Phillips C.[1], Fondevila M.[1], Carracedo Á.[1,3], Lareu M. V.[1]

[1] Forensic Genetics Unit, Institute of Forensic Sciences, Faculty of Medicine, University of Santiago de Compostela, Spain
[2] Department of Biomedicine and Prevention, Università degli Studi di Roma Tor Vergata, Rome, Italy
[3] Galician Foundation of Genomic Medicine, SERGAS, Santiago de Compostela

mafondevila@hotmail.com

Although a powerful and useful tool in forensic genetics, the genotyping of Single Nucleotide Polymorphisms (SNPs) has certain limitations due to their mainly bi-allelic variation. The reduced discrimination power per locus from two alleles is usually highlighted as a major drawback to forensic SNP analysis for identity purposes, but given the increased multiplexing capability of these markers, sufficient numbers of SNPs can be amplified in one PCR to achieve Random Match Probability (RMP) values equivalent to those of 15 STRs. Furthermore, another hindrance to adoption of SNPs in forensic casework is their limited ability to detect mixed-source DNA. In order to start to address both of the above issues, we have designed a SNaPshot™-based 29-plex genotyping assay of multiple-allele SNPs (28 tri-allelic and a single tetra-allelic SNP). The increase in variability from this type of SNP provides a higher discrimination power per locus and at the same time, this set has the ability to detect mixed DNA from the presence of multiple alleles in a profile. We selected the SNPs for maximum heterozygosity in three major population groups based on the most recent 1000 Genomes variant catalog for Europeans, Africans and East Asians. The fully optimized assay comprises a single-tube PCR followed by two parallel Single Base Extension reactions due to electrophoretic mobility

constraints associated with 3 or 4 allele peak positions per SNP. We present results from analysis of challenging casework DNA, including dilution series, highly degraded samples and simple two-way artificial mixtures. The increased discrimination power per marker is also outlined based on additional allele frequency data from HGDP-CEPH diversity panel genotyping.

194. EFFECTS OF STORAGE METHOD ON DNA DEGRADATION IN OLD BLOODSTAIN SAMPLES

Hara M.[1], Nakanishi H.[1,2], Takahashi S.[2], Tamura A.[3], Yoneyama K.[1], Saito K.[1,2], Takada A.1

[1] *Department of Forensic Medicine, Saitama Medical University, Saitama, Japan*
[2] *Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan*
[3] *Department of Legal Medicine, Osaka Medical College, Osaka, Japan*

ms2_hara@saitama-med.ac.jp

Introduction: When seeking a resolution to a long-term unsolved crime, STR genotyping of very old bloodstains is evidentially important. However, if specimen storage has been inappropriate, forensic data may be impossible to obtain. In the present study, we explored the effects of different storage methods on both DNA degradation and the results of preliminary blood tests.

Materials and Methods: Bloodstains stored at room temperature, 4°C, -20°C, and -80°C for 20 years, and whole blood samples stored at -20°C and -80°C for 20 years, were analyzed. DNAs were extracted and purified using EZ1 DNA Investigator Kits. DNA degradation ratios were assessed by calculating the ratios of DNA fragments 129 and 41 bp in length, and 305 and 41 bp in length, using KAPA Human Genomic DNA Quantification and QC Kits. Preliminary testing included leuco-malachite-green test, anti-human Hb testing via immunochromatography, and detection of the hemoglobin-beta (*HBB*) mRNA.

Results and Discussion: Bloodstains stored at room temperature were statistically degraded compared with fresh blood, as evidenced by the 129:41 bp and 305:41 bp fragment ratios.Bloodstains stored at 4C were statistically degraded compared with fresh blood, as evidenced by the 305:41 bp fragment ratio. All samples were positive upon leuco-malachite-green and anti-human Hb testing. *HBB* mRNA wasn't detected from whole bloods stored at -20°C and -80°C. Therefore, it is suggested that storing at below -20°C after making stain is better.

195. DTT QUENCHES THE PASSIVE REFERENCE SIGNAL IN REAL-TIME PCR

Norén L.[1], Sanga M.[1], Hedman J.[1,2]

[1] *Swedish National Forensic Centre, Linköping, Sweden*
[2] *Applied Microbiology, Lund University, Lund, Sweden*

lina.noren@polisen.se

Forensic DNA analysis is partly limited by PCR-inhibitory compounds present in the DNA extracts. Generally, these inhibitors disturb amplification, i.e. the production of amplicons. We have found that dithiothreitol (DTT) from the DNA extraction process can cause another type of real-time PCR disturbance, i.e. detection inhibition through fluorescence quenching. DNA extracts containing DTT substantially quenched the passive reference signal in the Quantifiler® HP DNA Quantification kit. This quenching resulted in overestimation of the DNA concentrations, as the target DNA signals are normalized to the passive reference signal. One negative control with no visible amplification plot was falsely given a high DNA concentration (22 ng/µL). Addition of increasing amounts of pure DTT to the qPCR reactions gradually decreased the passive reference signals. With 3.3 mM DTT in the reactions, corresponding to the amount of DTT transferred from the quantified DNA extracts, the passive reference fluorescence intensity was approximately 20% of the signal from samples without DTT. With 33 mM DTT the normalized fluorescence intensity decreased to approximately 3%. The hydrolysis probe fluorescence signals generated from amplification of target DNA templates were not quenched by DTT, indicating that DTT interacts directly with the passive reference dye (Mustang Purple). Purification of the extracts with Amicon Ultra filter devices

prior to quantification removed the inhibitory effect. The fluorescence quenching of DTT shows that PCR inhibition is not exclusively connected to amplification, but also to detection. Inhibition of real-time PCR detection may give unexpected effects such as the overestimation of DNA concentrations seen here.

197. THE REPRODUCIBILITY, ACCURACY & RELIABILITY OF LOW COPY NUMBER DNA PROFILING USING MULTIPLE SAMPLING OF PROFILES

Armstrong G.[1], Gunn P.[1]

[1] *Centre for Forensic Science, University of Technology Sydney, NSW Australia*

peter.gunn@uts.edu.au

Low Copy Number (LCN) DNA profiling involves a modification of the standard forensic PCR methods that was introduced to compensate for low levels of template DNA. At low-template inputs, DNA profiles often display stochastic effects such as extreme heterozygous imbalance, allele drop-in, and drop-out and exaggerated PCR artifacts such as exaggerated stutter peaks. These make the interpretation of profiles problematic.

One method developed by Gill et al[1] aims to overcome these challenges by dividing a template DNA sample into multiple aliquots, and combining the resulting amplifications into a "consensus profile." However there has been extensive discussion and critique of both the LCN method and of its interpretation, see for example ref [2]).

This study investigated the accuracy and reliability of the consensus approach. Two hundred and twenty consensus profiles were generated from low quantities (e.g. < 30 – 50 pg DNA) of a single source template, and were compared to a reference profile.

While only 66% of *individual* profiles could be confidently 'matched' to the reference profile, over 95% of the consensus profiles supports the contention that the "*consensus*" approach can be a reliable way of reconstructing a low template DNA profile.

1. Gill P. Application of low copy number DNA profiling. Croatian Medical Journal. 2001; 42(3):229-232.
2. Budowle B, Eisenberg AJ, Daal Av. Validity of low copy number typing and applications to forensic science. Croatian medical journal. 2009; 50(3):207-217.

198. TEST OF ESSPLEX_SE_QS WITH QUALITY SENSOR

Mogensen H. S.[1], Frank-Hansen R.[1], Morling N.[1]

[1] *Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

helle.smidt@sund.ku.dk

The ESSplex_SE_QS (Qiagen) amplifies sixteen STR loci including SE33 and Amelogenin. The reaction mix is supplemented with a set of quality sensors that are sensitive to inhibitors in the amplified samples. The quality sensors consist of a short fragment and a long fragment. Amplification of the long fragment is very sensitive to inhibitors while the short fragment is very robust to inhibitors. The kit sensitivity was tested with serial dilutions of high quality DNA. Full profiles were obtained down to 125 pg of DNA. The behavior of the quality sensors was investigated by adding various concentrations of Hematin or Indigo Carmine to high quality DNA samples. The quality sensor peak height ratio increased and the peak heights of the longer fragments of the profiles decreased with increasing concentration of inhibitor. The decreasing peak heights of the longer fragments are also a classical sign of DNA degradation. However, with artificially degraded DNA the quality sensor peak height ratio was not affected. Investigations of DNA extracts from stain samples from crime cases demonstrated the challenges with degradation and inhibition in the crime case lab. Several stains showing the typical sign of degradation with

decreasing peak heights of the longer fragments were actually inhibited as the peak height ratio of the quality sensors were greatly increased.

199. ENHANCED INTERROGATION FOR LOW TEMPLATE DNA SAMPLES

Benschop C.[1], Graaf E. S. M.[1], Sijen T.[1]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*

c.benschop@nfi.minvenj.nl

In forensic casework it is important to obtain as much information as possible from crime scene samples. Profiling results for samples with low-template (LT) amounts of DNA will improve when enhanced interrogation techniques, such as increased cycling or higher capillary electrophoresis (CE) injection settings, are applied. Although lower drop-out rates are obtained with enhanced profiling, this comes at the cost of exacerbated peak imbalance and drop-in. In this study, we examined the fold increase in peak heights when applying enhanced instead of standard profiling. Subsequently, we diluted DNA extracts for enhanced profiling in order to obtain DNA profiles that appear similar to standard profiling with respect to average peak height and percentage of drop-out. For instance, enhanced settings involved three or six times higher injection and the amount of DNA in the amplification were accordingly three or six times lower. All profiles were examined for peak height variation and type of drop-out. Furthermore, likelihood ratios (LRs) were computed using LRmix Studio and compared for profiles obtained using the various settings for PCR and CE. From the results we examined the robustness of enhanced interrogation techniques and assessed whether LR computations using enhanced profiles can be useful in casework practice.

200. A PANEL OF PCR-INHIBITORY REFERENCE MATERIALS FOR QUALITY EVALUATION OF MULTIPLEX STR ANALYSIS KITS

Sanga M.[1], Norén L.[1], Lindsten H.[1], Rådström P.[2], Ansell R.[1], Hedman J.[1,2]

[1] *Swedish National Forensic Centre, Linköping, Sweden*
[2] *Applied Microbiology, Lund University, Lund, Sweden*

malin.sanga@polisen.se

PCR inhibition is a key challenge in forensic DNA analysis. Substances interfering with the amplification of PCR products can lower the success rate of Short Tandem Repeat (STR) analysis or generate ambiguous DNA profiles. For forensic DNA laboratories it is therefore vital to have knowledge about how common inhibitory substances affect their STR analysis system. We have developed a broad-range panel of standardized PCR-inhibitory reference materials (RMs), representing the heterogeneous stains found at crime scenes. The panel, including solutions prepared from for example cigarette paper, chewing gum, moist snuff and humic acid, is a tool for quality evaluation of STR systems. We applied the RMs to challenge PowerPlex® ESX 16 Fast System (ESX Fast). Although ESX Fast tolerated high levels of some inhibitors, several RMs caused problems in different ways. Humic acid had a specific negative effect on amelogenin amplification, moist snuff hindered amplification of longer fragments, and chewing gum caused generally lowered allele peak heights. These different effects may provide information regarding the mechanisms of inhibition. For instance, our results indicate that one effect of humic acid on ESX Fast analysis is chelation of Mg^{2+} ions, thus altering the melting temperatures of the primers. In the developmental validation of STR systems, a limited evaluation of PCR inhibition involving only a few substances is generally performed. Applying a broad panel of RMs will ensure that a wider range of inhibitory substances from crime scene samples are included, giving a better understanding of inhibitor tolerance and effects.

201. EVALUATION OF DIRECT PCR IN CASES OF ALLEGED SEXUAL ASSAULT

Tobe S.[1], Swaran Y.[2], Dennany L.[3], Sibbing U.[4], Welch L.[3], Vennemann M.[4]

[1] Flinders University, School of Biological Sciences, Bedford Park, Australia
[2] Department of Chemistry Malaysia, Forensic Division, Jalan Sultan, Malaysia
[3] University of Strathclyde, Centre for Forensic Science, Glasgow, United Kingdom
[4] Forensische Molekulargenetik, Institut für Rechtsmedizin Münster, Münster, Germany.

Shane.tobe@flinders.edu.au

Sexual assault samples are some of the most common samples encountered in forensic analysis. These samples generally require a significant time investment due to differential extraction processes. We report on the first record of successful direct amplification of semen for STR analysis. Semen and blood were directly amplified successfully from denim, cotton and nylon (flesh toned stockings). Sample analysis showed complete profiles, for both semen and blood, that corresponded to the sample donor.

Further testing showed direct amplification of semen from GEDNAP samples and at dilutions ranging from 1:5 to 1:40. GEDNAP samples produced full profiles the same as those produced under standard differential extraction analysis. Full DNA profiles were reliably recovered from samples diluted 1:5. Samples at other dilution factors showed drop-out at various levels, which did not correlate to the dilution factor.

A differential isolation protocol was developed and successfully implemented to separate and directly amplify a mixture of semen and female epithelial cells. This study demonstrates the potential to incorporate direct PCR in cases of sexual assault to more rapidly obtain results and achieve a higher sensitivity.

202. CONCORDANCE STUDY OF FOUR DNA AMPLIFICATION CHEMISTRIES

Mason-Buck G.[1], Ballard D.[1], Lai C.[1], Syndercombe Court D.[1]

[1] Department of Pharmacy and Forensic Science, King's College London, London, United Kingdom

gabriella.r.mason-buck@kcl.ac.uk

Insourcing of forensic analysis allows laboratories to have greater autonomy in regards to the methods, reagents and suppliers that they use. As a result, amplification chemistry usage will differ between laboratories for a number of reasons, including; cost, time and product capabilities. It is therefore important that results obtained across laboratories are concordant to prevent incorrect results being obtained, evidence being missed and potential miscarriages of justice. DNA profiling capabilities have advanced with the new chemistries on the market and benefits include; reduced amplification time, increased marker range and improved Low Template Number analysis. In this study over 1200 DNA samples from 6 population groups (White British, Chinese, South Asian, North East African, West African and Middle Eastern) were analysed. All samples were amplified with the following four DNA amplification chemistires; PowerPlex® ESI 16 System (Promega), Investigator 24 Plex QS Kit® (Qiagen), GlobalFiler® Express (Life Technologies) and AmpFISTR® NGM SElect™ Express (Life Technologies). Concordance was verified across all chemistries and all samples exhibiting discordant results were sequenced.

203. INITIAL EXPERIENCE IN THE APPLICATION OF THE POWERQUANT SYSTEM AND THE ESSPLEX SE QS KIT FOR ANCIENT DNA ANALYSIS

Pflugbeil A. M.[1,2], Bruchhaus H.[1], Edelmann J.[1], Labudde D.[2], Dreßler J.[1], Thiele K.[1]

[1] Institute of Legal Medicine, Johannisallee DE-04103 Leipzig
[2] University of Applied Sciences – Forensic Science Investigation Lab (FoSIL), Technikumplatz 17, DE-09648 Mittweida

Anne-Marie.Pflugbeil@medizin.uni-leipzig.de

The successful application of commercial DNA quantification systems and

STR kits for ancient DNA (aDNA) down-stream analyses for forensic and anthropological purposes requires a continuous development of appropriate sensitive tools. Degradation-related poor quality of ancient DNA from long-time stored biological material such as bone from historical skeletal remains, often leads to a loss of information during molecular genetic characterization. A new generation of DNA quantification and STR kits consider mentioned challenges in analyzing aDNA by an optimized primer design combined with active master mix components. Against this background, the efficiency of the new PowerQuant™ (Promega) quantification system and the ESSplex SE QS STR kit (QIAGEN) for molecular genetic applications was tested. The analyzed sample batch consisted of aDNA isolates from skleletal remains of an excavation site from the early Middle Ages. Both analyses tools should be evaluated by their applicability with regard to highly degraded DNA as well as their sensitivity to display the presence of possible PCR inhibitors.

204. ANALYSIS AND INTERPRETATION OF DIFFICULT SAMPLES - THE INTERACTION OF A SUITABLE DNA QUANTIFICATION KIT WITH THE RIGHT STR ASSAY MAY FACILITATE PROCESSING OF CRITICAL TRACE MATERIAL

Di Pasquale F., Cornelius S., König M., Scherer M., Prochnow A., Peist R.

QIAGEN GmbH
Qiagenstrasse 1 40724 Hilden Germany

Mario.scherer@qiagen.com

In particular the analysis of challenging samples – low template, inhibited, degraded or any combination thereof – can benefit from quantification and STR assays that are well adapted to each other. Such workflows can ensure high first pass rates, but also assist in choosing appropriate rework strategies where necessary. Internal controls integrated into quantification as well as STR assays can be used to assess the quality of a sample and to monitor if amplifications work properly.

The Investigator Quantiplex HYres assay uses a multi copy target to provide sufficient sensitivity to allow a good correlation to STR results even at the lower end. A zero quant obtained is very likely corresponding to no STR profile. The internal control of the quantification indicates potential inhibition. It has been designed to respond to inhibition before the quantification itself is affected, but to be robust enough to make predictions on very tolerant STR assays, as the Investigator 24plex QS kit. However, in particular due to variable template input amounts used for the STR assay, this prediction can never be perfect. This prompted us to integrate an internal performance control, the so-called Quality Sensor (QS) into STR assays as well. Using this advanced Quality Sensor allows deriving unambiguous information on inhibition or degradation to better interpret the STR result and to select the most appropriate strategy for further processing of the sample.

We show data on case studies to illustrate a more efficient and cost-effective laboratory workflow based on the combined use of these assays.

205. SYSTEMATIC STUDY ON THE ANALYTICAL PARAMETERS RELEVANT TO ACHIEVE RELIABLE STR PROFILES, AS ASSESSED IN A MULTICENTRE DATA SET

Marrubini G.[1], Previderè C.[2], Sorçaburo Cilieri S.[3], Grignani P.[2], Pitacco P.[3], Consoloni L.[3], Fattorini P.[3]

[1] Department of Drug Sciences, University of Pavia, Italy
[2] Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy
[3] Department of Medicine, Surgery and Health, University of Trieste, Italy

fattorin@univ.trieste.it

A recent report on a collaborative exercise of the ISFG Italian Working Group involving 25 laboratories was focused on the quantification of an artificially

damaged DNA and the resulting definition of the STR profile (Fattorini, Electrophoresis 2014). The results showed that the performances of the laboratories were characterized by a relevant dispersion of the data and by a high incidence of PCR artifacts. The results collected from the study allowed to understand that the performances of the laboratories depended more on the operating conditions chosen than on the nature of the sample processed. The data however could not be used to model the results and predict to which extent changes in the operating analytical conditions affected the methods sensitivity and accuracy in the quantification and STR profiling of the damaged DNA. Therefore a new study was started using the same damaged DNA sample and involving two labs which used the most commonly selected kit (*IdentifilerPlus*) and sequencer (*ABI 310*). Sixteen experiments were planned using a Box-Behnken experimental design and were carried out in each laboratory. The aim of this study was to model and describe the role of the template amount, number of amplification cycles, volume of the PCR reaction, and sample dilution prior to capillary electrophoresis in determining the assay sensitivity, the profiling accuracy and the occurrence of PCR artifacts. The results obtained were used to discuss the role of these factors in the outcomes of the collaborative study.

206. INSERTION OF AMELOGENIN MARKER IN THE 38 HID-INDELS MULTIPLEX REACTION

Braganholi D.[1], Polverari F.[1], Ambrósio I.[2], Cicarelli R. M. B.[1,2]

[1] *Chemistry Institute, Unesp, Araraquara, Brasil*
[2] *Faculty of Pharmaceutical sciences, Unesp, Araraquara, Brasil*

danilobraganholi@hotmail.com

Pereira et al. (2009) reported a method for multiplex analysis of a set of 38 INDELs autosomes known polymorphic in different populations of the world, which can be used in the identification of individuals but this methodology not originally introduced marker for the analysis of amelogenin. In laboratory of paternity of UNESP/Araraquara, Brazil, kinship analysis are performed using samples with degraded DNA, where the amplification of 38 HID-INDELs was efficient compared to STRs methods in these samples. For paternity tests also analyze STRs, and the verification of the sex of samples is done by the amelogenin marker analysis present in commercial kits, but the lack of ampification of STRs may interfere in the correct reading of amelogenin in the electropherogram. Trying to solve this problem we decided to add the amelogenin marker in multiplex reaction with 38 HID-INDELs. Through literature search and *in silico* analysis we select the pair of primers described by Krenke et al. (2002), and we made a small alteration in the sequence of the reverse primer, for the amplification of a fragment with 6 pb additional for correct reading of the amelogenin marker and 38 HID-INDELs in the electropherogram, allowing the confirmation of the sex of samples. Until this moment, the multiplex reaction 38 HID-INDELs with amelogenin marker was used in the analysis of fifteen cases of biological kinship with degraded DNA samples helping to solve them.

1. Pereira R, Phillips C, Alves C, Amorim A, Carracedo A and Gusmao L: A new multiplex for human identification using insertion/deletion polymorphisms. Electrophoresis. 2009; 30: 3682-3690.
2. Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, Tomsey CS, ZachettiJM, Masibay A, Rabbach DR, Amiott EA and Sprecher CJ: Validation of a 16-locus fluorescent multiplex system. J. Forensic Sci. 2002; 47(4): 773-785.

207. DNA FROM STAINS OF SEMEN AND SEMINAL FLUID PRE AND POSTVASECTOMY RECOVERED AFTER 10 YEARS OF STORAGE

Mautoni C.[1], Sousa M. L. D. A. P. O.[2], Silva M. S.[1], Soares-Vieira J. A.[3], Bittencourt E. A.[1], Astolphi R. D.[1], Iwamura E. S. M.[1]

[1] *Department of Pathology , Federal University of Sao Paulo, Sao Paulo, Brazil*
[2] *Center for Biology and Biochemistry, Institute of Criminology of Sao Paulo, Sao Paulo, Brazil*

[3] Department of Legal Medicine, University of Sao Paulo, Sao Paulo, Brazil

Vasectomy is a widely used method of contraception. The absence of spermatozoa in the ejaculate of azoospermic individuals increases the difficulty of extracting DNA for analysis, especially if the biological samples are in the form of spots and stored for years. The aim of this study is to evaluate the possibility of obtaining autosomal and Y-STR profiles from stains containing the ejaculate of individuals pre and postvasectomy, stored in cotton fabric for a period of 10 years. Samples from 28 (twenty eigth) individuals: semen prevasectomy and seminal fluid (postvasectomy) stains, stored from 2004 to 2014 at room temperature were used. Three cutouts of 1.5 cm in diameter (punch) were taken from central region of each spot. It was used the QIAamp DNA Mini kit (Qiagen) to extract DNA with an adapted protocol followed by quantification by Nanodrop equipment. Genetic profiles were obtained using ESI 17 PowerPlex® (Promega) . Capillary electrophoresis was performed on the ABI PRISM 3500XL DNA Analyzer. The DNA extraction was successful in all punch analyzed, and its concentration ranged from 1.1ng/uL to 23.3ng/uL in the prevasectomy and from 0.8ng/uL to 7 ng/uL in postvasectomy punch. The samples had suitable purity, reflecting the presence of few contaminants. Currently, the techniques of DNA amplification are extremely sensitive and capable of assessing human DNA in small quantities generating partial or full profiles, trespassing situations such as long storage periods in cotton fabric. These data can be used as a benchmark for forensic analysis in cases of semen analysis.

208. DNA GENOTYPING ANALYSIS OVERVIEW: PROFILE OF RAPE CASES IN THE STATE OF PERNAMBUCO, BRAZIL

Oliveira N.[1], Ekert M.[1], Souza C.[2], Almendra K.[2], Santos S.[3], Lima-Filho J.[1,4]

[1] Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Recife-Brazil
[2] Laboratório de Perícia e Pesquisa em Genética Forense, Estado de Pernambuco, Jaboatão dos Guararapes-Brazil
[3] Gerência Geral de Polícia Científica, Secretaria de Defesa Social do Estado de Pernambuco, Recife – Brazil
[4] Departamento de Bioquímica, Universidade Federal de Pernambuco, Recife –Brazil

nataliacybelle.89@gmail.com

Identification of perpetrators is crucial in criminal investigations, mainly in rape cases. Analysis of the area, victims' profile, as well as genotyping DNA markers from rapists may help to determine hotspots of this crime, in a way to prevent its recurrence. As there is a lack of this type of study in Northeastern Brazil population, herein we report genetic data analyses from rape cases that occurred in Pernambuco from 2012 to 2014. Also, the distribution of the cases, as well as a general profile of the victims were analyzed. Vaginal, anal and other body fluids samples were used in this study, obtained in cases from Laboratório de Perícia e Pesquisa em Genética Forense (LPPFG-PE). The results showed that 61% of the cases are mainly located in the metropolitan area of Pernambuco. Eighty-six percent of the victims were females and 35% of them were under age. Allele frequency was determined by at least 16 STRs and, in some cases, by Y-STRs and InDel markers. This study may contribute to future efforts to elucidate more accurately rape crime.

1. Z. Jakovski, R. Jankova, A. Duma, B. Janeska, G. Pavlovski, D. Marjanovic, Forensic approach to analyzing rape cases, Forensic Sci. Int. Genet. Suppl. Ser. 4 (2013) e45–e46.
2. E.Y. Lee et al. Analysis of 22 Y chromosomal STR haplotypes and Y haplogroup distribution in Pathans of Pakistan., Forensic Sci. Int. Genet. 11 (2014) 111–6.
3. A. Oliveira et al., Male lineage strata of Brazilian population disclosed by the simultaneous analysis of STRs and SNPs., Forensic Sci. Int. Genet. 13 (2014) 264–8.
4. M. Andersen et al. Identifying the most likely contributors to a Y-STR mixture using the discrete Laplace method. Forensic Science International: Genetics 15 (2015) 76–83.

209. EASY AND FAST PROCEDURE TO ISOLATE, PURIFY AND IMMORTALIZE DNA FRAGMENTS FOR ALLELIC LADDERS CONSTRUCTION

Burgos Figueroa G.[1,2], Restrepo Arango T.[2], Ibarra Rodríguez A.[2], Gaviria A.[3], Machado Rodríguez G.[2,4], Mora C.[5], del Pilar Lizarazo Quintero R.[5]

[1] *Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito, Ecuador*
[2] *Grupo de Investigación Identificación Genética IdentiGEN, Universidad de Antioquia, Medellín – Colombia*
[3] *Laboratorio de Genética Molecular, Cruz Vital - Cruz Roja Ecuatoriana, Quito – Ecuador*
[4] *Grupo de Investigación de Biotecnología, Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Medellín – Colombia*
[5] *Laboratorio de Genética, Instituto Nacional de Medicina Legal y Ciencias Forenses, Bogotá – Colombia.*

anibalgaviria@hotmail.com, longmantrainer@gmail.com

Allelic ladders are used both in validation and routine casework for human STRs. These are obtained by mixing individual genotypes. However, when alleles are obtained from heterozygous individuals, they cause allelic unbalance in the ladder mix and dependence upon original DNA extract availability. In this work we separated amplified alleles from heterozygous state by agarose or Polyacrylamide gel electrophoresis. Target alleles were recovered by gel puncturing and then reamplified using punctured DNA as template. Dilutions (from 10^{-3} to 10^{-4}) of these amplificates were used as new templates for PCR with fluorolabelled primers. This strategy was successfully used to obtain allelic ladders for mini STRs D10S1248 and D14S1434 tetra nucleotides which conform NC01 triplex with D22S1045, used for degraded DNA analysis. It is also worth noticing that this procedure could also be useful if new or mutant alleles are to be sequenced.

1. Bär W., Brinkmann B., Budowle B., Carracedo A., Gill P., Lincoln P., Mayr W., Olaisen B: DNA recommendations. Further report of the DNA Commission of the ISFG regarding the use of short tandem repeat systems. Forensic Sci Int. 1997; 87(3): 179-4
2. Kwok WT, Miu YWM, Cheuk YL: Effects of agar and agarose on Vent DNA polymerase and Taq DNA polymerase: Biotechnology letters 1995, vol 17, No1, pp. 1-6.
3. Coble MD & Butler JM: Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA. J. Forensic Sci. 2005, 50: 43-53.
4. Butler JM & Coble MD: Authors' response. J. Forensic Sci. 2007, 52: 494.

211. ON THE USE OF THE DNASCAN RAPID DNA ANALYSIS SYSTEM (GE HEALTHCARE)

Vilela Lamego C.[1], Keyser C.[1,2], Ludes B.[2,3]

[1] *INTS, Paris, France*
[2] *Institute of Legal Medicine, Strasbourg University, Strasbourg, France*
[3] *Institute of Legal Medicine, Paris, France*

STR analysis is the reference method for human identification and kinship testing in forensic sciences. Current STR typing involves highly trained forensic analysts who use multiple instruments to process and analyze samples in a laboratory environment. This conventional DNA processing takes hours to days. In recent years, advances in DNA technology have led to the development of faster and more efficient processing techniques. One of them has been to integrate the entire workflow processing samples from buccal swabs to DNA profiles. DNA identification using such fully integrated systems has been termed Rapid DNA. This work describes a trial intended to evaluate the efficacy of one of them, the DNAscan Rapid DNA Analysis System developed by GE Healthcare and NetBio. This system provides a fully automated process (from sample collection to DNA results) taking less than two hours. As part of a pilot study to test the quality of the DNA profiles obtained with this system, buccal samples were collected from 57 volunteer donors using NetBio cotton-swabs. The data obtained demonstrates that the DNA scan system produces high quality results with a success rate of producing full profiles for buccal samples

of 96,5% (55/57) and that the quality of the profiles is high since few stutter, peak height imbalance or artifact were observed.

212. PRE-AMPLIFICATION AS A TOOL FOR RESOLVING MIXED DNA PROFILES

Leonov S. N.[1], Zhernovkov V. E.[1], Orekhova N. N.[1], Skoromyslova E. V.[1], Orekhov V. A.[1]

[1] *GORDIZ Ltd, Moscow, Russia*

orekhov@gordiz.ru

Mixed DNA profiles are a common feature in the forensic DNA analysis of questioned evidence samples. Methods for attempting to physically separate contributors from sexual assaults, i.e., sperm and epithelials, are well established. Mixed DNA profiles with clear major and minor components can also be analyzed to try and deduce the individual contributors. However many mixed samples do not yield to such methods.

Here we outline a molecular biological approach to mixture deconvolution that takes advantage of the possible differences in DNA quality (i.e., degree of DNA degradation) of the different contributors in a mixture. These differences can be accentuated by performing a short, 5-10 cycle, pre-amplification of the extracted and purified evidentiary DNA. Unlabeled PCR primers located 100-200 bp upstream from labeled STR primer binding sites were used in the pre-amplification. The amplified DNA from the pre-amplification was then used as a template for standard multiplex DNA-STR amplification. By comparing the shifts in peak heights from the DNA profiles obtained before and after pre-amplification, it has been possible to deduce the DNA profiles of the individual contributors.

The DNA from the contributor with less degradation is preferentially amplified and tends to become the major profile after pre-amplification. Results from laboratory prepared and real-world samples are demonstrated.

213. REDUCED PCR CYCLING TIME AMPLIFICATION USING AMPFℓSTR IDENTIFILER KIT

Iyavoo S., Wolejko A., Furmanczyk D., Graham R., Myers R., Haizel T.

Anglia DNA Services Limited, Norwich, United Kingdom

s.iyavoo@angliadna.co.uk

Anglia DNA Services Limited uses the AmpFℓSTR® Identifiler® kit (Life Technologies™) for paternity, maternity and relationship testing. Generation of a DNA profile at Anglia DNA involves DNA extraction, PCR amplification and capillary electrophoresis in a process that takes approximately 8 hours. Of this time, 3 hours are attributed to the PCR amplification step. It is therefore important to reduce this time which will lead to a considerable benefit of offering faster service to the customers. Many publications have recommended fast PCR method for AmpFℓSTR® Identifiler® kit, with the addition of new buffers and new enzymes. However, this study was carried out without any addition of buffers or enzymes and by altering the time period of several PCR conditions especially the final extension time. Tested samples were also amplified using manufacturer's recommended standard PCR cycling parameters for comparison study. There was no significant difference in the quality of the profiles produced in the reduced PCR cycling time amplification method compared to those produced in the standard PCR method. The application of this reduced PCR cycling time amplification resulted in saving almost 1/4 of the time needed for PCR amplification without compromising the quality of the profiles.

214. REDUCED VOLUME PCR AMPLIFICATION USING AMPFℓSTR IDENTIFILER KIT

Iyavoo S., Cummings S., Wolejko A., Furmanczyk D., Graham R., Myers R., Haizel T.

Anglia DNA Services Limited, Norwich, United Kingdom

s.iyavoo@angliadna.co.uk

AmpF*ℓ*STR® Identifiler® kit (Life Technologies™) is used by Anglia DNA Services Limited for paternity, maternity and relationship testing. The manufacturer's recommended PCR volume for this kit is 25 µl though samples have been amplified successfully with this kit using a PCR volume of 12.5 µl. Further reduction in reaction volume from 12.5 µl to 10 µl would offer a considerable benefit due to cost-saving but it should be without compromising the quality of the results. For comparison study, the tested samples were amplified in both 12.5 µl and 10 µl PCR volumes. Statistical calculations were carried out on the produced profiles and the results were compared based on the profiles concordance, amplification efficiency and the profiles quality. The profiles of the tested samples amplified in a volume of 10 µl showed 100% concordance with the same samples amplified in a volume of 12.5 µl. Also, there was no significant difference in the quality of the profiles produced in a reaction volume of 10µl compared to those produced in a reaction volume of 12.5µl. The application of this reduced volume PCR amplification would represent an additional 20% cost saving on the reagents.

215. IS IT POSSIBLE TO USE FORENSIC DNA PHENOTYPING IN BRAZILIAN POPULATION?

Fridman C.[1], Cardena M.[1], Felícia L.[1], Gonçalves F.[1]

[1] *Department of Legal Medicine, University of São Paulo, Medical School, Brazil*

cfridman@usp.br

The prediction of human traits in order to help forensic investigations has been one of the most interesting researches in the last decade. Some pigmentation genes such as SLC45A2 variants have been associated with phenotypic diversity of skin, eyes and hair color in homogeneous populations. The aim of this study was evaluated the possibility of association between 3 *SLC45A2* polymorphisms (rs26722, rs16891982 and rs2278007) with skin, eye and hair color in a sample of 598 individuals of admixed population from Brazil, intending to use it in forensic genetic situations. DNA sequencing was performed with BigDye Terminator v3.1 and capillary electrophoresis was performed in ABI3130. Presence of rs16891982 variant (C>G) was associated with non-black skin (OR 16.35; CI 6.014-44.5), as well as with non-black hair (OR 18.12; CI 5.25-62.6) and light eyes (OR 5.04; CI 2.6-9.6). Polymorphism rs2278007 in heterozygous (AG) was associated with a lower probability of individual presenting lighter pigmentation of skin and hair. Our data corroborate the findings of other studies in homogeneous populations, suggesting that the analysis of *SLC45A5* polymorphisms can be used as a tool to access some phenotypic traits and use to help forensic identifications as molecular predictor of phenotypes also in admixed populations. These results supplement the previous ones we showed in the same population with the genes *SLC24A2* and *ASIP*, and they are part of a major project which aims to study the correlation of several pigmentation genes and skin, hair and eye color in Brazilian population. Supported: FAPESP, HC-LIM40

216. EVALUATION OF THE CHELEX-100 METHOD AND FOUR COMMERICAL DNA EXTRACTION SYSTEMS

Idris B.[1], Goodwin W.[1, 2]

[1] *School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, UK*
[2] *School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, UK*

thebush23@hotmail.com

Routinely used extraction methodologies rely on organic substances, chelating agents or silica-based capture. Recently the use of automated bench-top technologies has gained popularity in the forensic community. In this study we compared 5 methods to efficiently extract DNA from 3 different body fluid types (blood, semen and saliva). Methods tested were: the Chelex®-100,

the QIAamp® DNA Investigator Kit (Qiagen, Germany) and InnuPREP® forensic extraction kit (Analytikjina, Germany) in addition to two automated extraction systems; the EZ1® system (Qiagen) and The AutoMate Express™ System (Applied Biosystems). Evaluation was based on the extracted DNA quantity and quality using real-time quantification (Quantifiler® Human, Applied Biosystems). Additional criteria such as consistency, ease of use and cost efficiency were also evaluated. Both the AutoMate Express™ System and the Chelex-100 method showed the highest DNA yield. All extraction methods yielded good quality DNA with an IPC value between 26 Ct and 28 Ct. Analysis Of Variance (ANOVA) showed significant differences between the methods tested (p-value= 3.8e-15 for blood, 3.47e-10 for semen and 2.82e-10 for saliva). Standard Deviation (SD) calculation (8 repeats per body fluid) showed that the EZ1 system was the most consistent, whereas both the Chelex-100 method and the AutoMate Express™ System showed a high level of inconsistency in their DNA yield. Although the AutoMate Express™ System was the most favorable method tested, the chelex-100 method seemed a much affordable alternative which does not compromise on DNA yield or quality.

217. SUCCESSFUL DIRECT STR AMPLIFICATION OF ANAGEN HAIR FOLLICLES AFTER NUCLEAR STAINING

Haines A. M.[1], Tobe S.[1], Kobus H.[2], Linacre A.[1]

[1] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[2] *School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia*

alicia.haines@flinders.edu.au

Hairs are commonly encountered at a crime scene through natural shedding (telogen) or trauma such as during an assault (anagen). The forcible removal of anagen hairs results in retention of the root sheath and therefore the potential for DNA evidence. Telogen hairs lack a root sheath and do not provide a good source of DNA. Microscopy may be performed on all hair samples to detect whether there are cells adhering to the proximal tip to determine if there is a chance of success from subsequent DNA profiling. To aid in improving the microscopy, we report of the staining of hair roots using a range of dyes (SYBR® Green I, Diamond™ Nucleic Acid Dye, GelGreen™, EvaGreen™ and Redsafe™) and subsequent results from DNA profiling. Results showed that nuclei were visualized using all the dyes except for GelGreen™. The hairs were then directly amplified and all samples produced an STR profile that met requirements for uploading to a DNA database. The staining procedure conducted before direct amplification had no or little effect on the PCR and electrophoresis of the STR fragments. These results show that these nucleic acid binding dyes can be used as a preliminary assessment as to the viability of the sample (number of nuclei present) for STR analysis.

218. DURATION OF IN SITU FLUORESCENT SIGNALS WITHIN ANAGEN HAIRS

Haines A. M.[1], Tobe S.[1], Kobus H.[2], Linacre A.[1]

[1] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[2] *School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia*

alicia.haines@flinders.edu.au

Nuclei within anagen hair follicles can be viewed using fluorescent microscopy after staining with a nucleic acid binding dye. Anagen hairs are still in the growth phase and generally only removed by force such as pulling, plucking or trauma and can be important evidentiary items at a crime scene. Staining of the hairs can be a fast, easy, method to determine if hair samples have DNA present making it worthwhile to attempt to obtain a DNA profile for evidentiary purposes. SYBR® Green I and Diamond™ Nucleic Acid Dye are two such dyes. The duration of the in situ fluorescent signal of both these nucleic acid dyes was studied to determine how long the samples can be kept in storage after staining yet still be capable of producing a fluorescent signal. Our results show that when staining with Diamond™ Nucleic Acid Dye the fluorescent signal could be viewed months after initial staining period. However when the hair was stained with SYBR® Green I there was a significant reduction in

the fluorescent signal within 6 days of initial staining. Our conclusion is that Diamond™ Nucleic acid dye was a viable dye for staining hair and keeping the samples in storage for later analysis.

219. COMPARATIVE STUDY OF TWO DNA EXTRACTION METHODS IN DIFFERENT TISSUES AND CONDITIONS OF DEGRADATION

Jakovski Z.[1], Jankova Ajanovska R.[1], Stankov A.[1], Pavlovski G.[1], Poposka V.[1], Marjanovic D.[2, 3]

[1] Institute of Forensic Medicine and Criminology, School of Medicine, University "Ss. Cyril and Methodious", Skopje, Macedonia
[2] Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina
[3] Institute for anthropological Research, Zagreb, Croatia

zlatedr@yahoo.com

The aim of this study is to compare two methods of extraction of DNA from different tissues (spleen, liver, lungs, kidney, heart, muscles, and pancreas) with Ph-CL and QIAAamp® DNA Mini kit and to confirm which of these two methods provide better results. Degradation of the tissues was in 3 controled conditions in a period of 6 months: room temperature, in a refridgerator at +4 and outside temperature in the period 2.3-17.8.2010. In total, 210 analyses were performed and 20-30 mg of tissue was taken. Quantification was performed with Quantifiler kit on 7500 RealTime PCR ABI and analysed with 7500 System SDS version 1, 2, 3 software. The temperature was continuosly measured with digital TESTO termomethers on each 2 hours. T-test was used to compare the two extraction protocols from tissues. Comparation of different tissues which were degradated in 3 controled conditions and extracted in 5 periods was performed with ANOVA. This study shows that bigger yield of DNA from the tissues can be isolated with PhCl extraction method, but, also, with QIAAamp® DNA Mini kit, eaven though less amount of isolated DNA is obtained, it can be used for further PCR reaction. PhCl method is slow, cancerogenous, more expenisve and the large amount of DNA can make a problem in the amplification process. Comparative results from different tissues show that there is no big diference in the extracted yeld of DNA. We proved that in putrefied tissues non toxic and fast method can be used for DNA extraction. Our study shows that the isolated DNA does not decerase with the increase of PMI and ambiental temperature, most probably due to the short decay period of 6 months that we were analysing. However, in forensic DNA analysis we should take into account that the quality and quantity of extracted DNA depends on the ambiental conditions, PMI and the type of tissue. The fastest degradation happens in tissues which have more proteolotyc enzymes.

1. J Burger, S Hummel, B Herrmann, W Henke, DNA preservation: A microsatellite-DNA study on ancient skeletal remains, Electrophoresis, (1999) 20, 1722-1728
2. F.A. Kaestle, K.A. Horsburgh, Ancient DNA in Anthropology: Methods, Applications, and Ethics, Anthropology, (2002) 45:92-130
3. P.M. Shneider, K. Bender, W.R. Mayr, W. Parson, B. Hoste, R. Decorte et all, STR analysis of artificially degraded DNA-result of a collaborative European exercise, Forensic Sci Int. 139 (2004) 123-134
4. H. Rompler, P.H. Dear, J Krause, M. Meyer, N. Rohland, T. Shoneberg, H Springgs, M Stiller, M. Hofreiter, Multiplex amplification of ancient DNA, Nature Protocols, 1 (2006) 2:720-728
5. L. Rubio, L.J. Martinez, E Martinez, S. Martin, Study of short-and long term storage of teeth and its influence on DNA, J Forensic Sci 54 (2009) 6:1411-1413
6. D.H. O'Rourke, M.G. Hayes, S.W. Caryle, Ancient DNA studies in physical anthropology, Annu. Rev. Anthropol. (2000) 29:217-42
7. S Paabo, H. Poinar, D Serre, V.J. Despres, J. Hebler, N. Rohland et all, Genetic analyses from ancient DNA, Annu. Rev. Genet. (2004) 38:645-79
8. C. Curts, B. Madea, Full DTR profile of a 67-yeat old bone found in a fresh water lake, J Forensic Sci Jan (2011) 56:172-175

9. S. Paabo, Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification, Proc. Nat. Acad. Sci. USA (1989) 86:1939-43
10. R. Higushi, rapid, efficient, DNA extractions for PCR from cell or blood, Amplification, (1989) 2:1-3
11. M. Allen, A.S. Engstrom, S. Meyers, O Handt, T. Saldeen et all, Mitochondrial DNA sequencing of shed hairs and saliva on robery caps: senzitivity and matching probabilities, J Forensic Sci (1998) 43:453-64
12. L.A Johnson, J.A. Ferris, Analysis of postmortem DNA degradation by single-cell gel electrophoresis, Forensic Sci. Int. 126 (2002) 1:43-47
13. D. Sanoudou, P.R Kang, J.N. Haslett, M. Han, L.M. Kunkel, A.H. Beggs, Transcriptional profile of postmortem skeletal muscle, Physol. Genomics (2004) 16:222-228
14. M.A El-Harouny, A. El-Dakroory, S.M. Attalla, N.A. Hasan, S.E. Hassab El-Nabi, The relationship between postmortem interal and DNA degradation in different tissue od drowned rats, J. Forensic Med. Clin. Toxicol. XVI (2008) 2:45-61
15. S. Leonard, J. logel, D. Luthman, M. Cova, D. Kirch, R. Ereedman, Biological, stability, of mRNA isolated from human postmortem brain colections, Biol. Psychiatry, 33 (1993) 6:456-466

220. THE END OF BAD HAIR DAYS

Handt O.[1,2], Linacre A.[2]

[1] *Forensic Science SA, Adelaide, Australia*
[2] *Flinders University, School of Biological Sciences, Bedford Park, Australia*

Shed hairs are commonly encountered at crime scenes, yet consistently obtaining DNA profiling results from them has traditionally been difficult. In this current study a direct PCR approach was used to obtain DNA profiles from hairs using the GlobalFiler (GF) DNA profiling kit (Life Technologies). Initially, only anagen hairs were added as template for amplification. 30% of the obtained DNA profiles were of appropriate quality for interpretation. The remainder had artefacts associated with excessive amounts of DNA such as split peaks, gross peak height imbalances between loci and stutter of stutter.

Prep-n-Go™ buffer (Life Technologies) has been developed for direct PCR amplification of non FTA reference samples to produce reproducible and consistent results. The addition of Prep-n-Go™ buffer to reaction tubes containing hair samples substantially increased amplification success and profile quality. Profiles were obtained for 100% of anagen hairs tested and more than 50% resulted in complete GF profiles. Similarly, direct amplification of telogen hairs with addition of Prep-n-Go™ buffer increased the success of obtaining DNA profiles and also improved inter-locus peak balance. We conclude that setting up direct PCR tests can be an effective approach to obtain DNA profiles from anagen and telogen hair samples left at crime scenes and that the addition of Prep-n-Go™ buffer might be beneficial for other crime scene samples if a direct PCR approach is taken.

221. EVALUATION OF THE RAPIDHIT 200 SYSTEM: A COMPARATIVE STUDY OF ITS PERFORMANCE WITH MAXWELL DNA IQ / IDENTIFILER PLUS/ABI 3500XL WORKFLOW

Thong Z.[1], Phua Y. H.[1], Loo E. S.[1], Goh S. K.[1], Ang J.[1], Looi W. F.[1], Syn C.[1,2]

[1] *DNA Profiling Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078, Singapore*
[2] *Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore*

thong_zhonghui@hsa.gov.sg

The RapidHIT™M System was designed for the processing of reference samples from extraction through to capillary electrophoresis within two hours. This function has been successfully demonstrated in various laboratories worldwide. The present study evaluated the performance of the RapidHIT™

System in handling crime casework samples with respect to reproducibility, contamination, sensitivity, versatility and the possibility of sample re-extraction. The results indicated that the RapidHIT™ System could generate high quality DNA profiles comparable to those from the standard protocol of Maxwell® 16 DNA IQ™ System, Identifiler® Plus and ABI 3500xL. Results also showed that the instrument could generate DNA profiles from samples containing lower amounts of DNA (0.5 µl of blood) albeit with more allele and locus dropouts when compared to the standard protocol. The ability to process blood swabs, blood-stained FTA punches, semen swabs, buccal swabs, muscle, bone marrow, fingernail clippings and cigarette butts at a good success rate indicated the versatility of the RapidHIT™ System. Of particular significance was the potential to recover additional alleles via re-analysis of the samples retrieved from the RapidHIT™ cartridge. Preliminary studies on pre-treated samples containing trace DNA have also suggested improved recovery of alleles. In summary, our results showed that the RapidHIT™ System was able to process casework samples for the purpose of providing rapid intelligence through DNA database searches and reference matching. Confirmative DNA results can be obtained through either concurrent processing of duplicate samples via standard protocol or re-extraction of samples retrieved from the RapidHIT™ sample cartridge.

222. REBALANCING OF DEGRADED DNA PROFILES THROUGH PREFERENTIAL EXTRACTION

Baptista L. V.[1,2], Rattanarungruang N.[1], Goodwin W.[1]

[1] *School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, UK*
[2] *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, Brasil*

LVBaptista@uclan.ac.uk

After death DNA becomes progressively more fragmented as biological tissue degrades and this results in a decreasing ability to gain a complete STR profile. When extracting and profiling DNA from human remains understanding the likely persistence of DNA in different tissues is important. Studies in the UK have demonstrated that when using pigs as an experimental model, DNA up to 400 bp will persist for up to 3 weeks. However, it is well known that DNA degradation, especially in muscle tissue that has not become dehydrated, is dependent to a large degree on temperature. To assess DNA persistence in more extreme environmental conditions pig carcasses were exposed to the environment in Thailand during June for 1 week, with samples being collected every 12 h. In field conditions muscle DNA could not be amplified after 36 h exposure, unless the muscle was collected from tissue that was in contact with the ground; DNA persisted for 72 h in these samples.

223. EVALUATION OF DNA EXTRACTED FROM UP TO 16 YEARS OLD POST-MORTEM BLOOD FTA CARDS USING QUANTIFILER HUMAN PLUS (HP) QUANTIFICATION KIT

Rahikainen A. L., Palo J., Sajantila A.

Department of Forensic Medicine, University of Helsinki, Helsinki, Finland

anna-liina.rahikainen@helsinki.fi

Post-mortem studies, which often harvest the extreme-cases from the population, offer unique opportunities for genetic research. At our department, blood sample from each autopsy has been taken routinely on FTA™ Gene Cards since 1998. DNA on these cards should be stable and preserved for at least 17 years. The aim of this study was to evaluate the effect of FTA storage time on DNA quality and quantity.

Four random samples from eight time points covering sampling years 1998 to 2013 (n = 32) were collected and extracted with Automate *Express*™ System using PrepFiler *Express*™ Forensic DNA Extraction Kit. Extracted DNA was quantified and qualified with Quantifiler HP Quantification kit. The effect of degradation on the amplification was evaluated with short tandem repeat (STR) multiplex assay, and by amplifying a large *CYP2D6* gene in long PCR

reaction.

Our results show, expectedly, negative correlation between storage time and DNA quantity and clear increasing trend in degradation indices with time. This manifested lucidly in the STR-profiles although full profiles were obtained from all samples. The effect was even more apparent in the 5.1 kb *CYP2D6* gene. This difference on amplification success is due to the amplicon length, which is much smaller in the forensic STRs (<500 bp) than in the *CYP2D6* (5.1 kb).

According to our results, DNA on FTA cards is rather stable over long time. However, the DNA quality and quantity is diminished over the years and when planning the experiments, especially with longer PCR amplicons, this should be taken account.

224. ANALYSIS OF AGED SEMINAL STAINS BY CURRENT FORENSIC DNA APPROACH

Bini C.[1], Ceccardi S.[1], Trento C.[1], Carano F.[1], Pelotti S.[1]

[1] *Department of Medical and Surgical Sciences, Legal Medicine, University of Bologna, Bologna, Italy*

susi.pelotti@unibo.it

In sexual assault crimes, the finding of seminal stains and their correct storage are crucial for forensic investigations. Actually, undetected ‚cold' cases involving sexual assault can be solved decades after investigations by analysing DNA from stored evidences (1). Recently, during restoration work of the museum in the Institute of Legal Medicine of the University of Bologna, three pieces of cotton fabric were found within an old envelope of the Institute showing handwritten "seminal stains". It was estimated that the seminal stains date back to the first half of the '900, when the institute had been transferred in the today's site and Baecchi's staining were already used. The aim of this study was to analyse these aged seminal stains by current forensic methods including the semenogelin test, microscopic identification of spermatozoa, autosomal, Y chromosome and X chromosome STRs, and mitochondrial DNA analysis. The use of Y-chromosome analysis by Y-STRs kits, and the new RM Y-STRs are suitable in rape casework also for predicting population of origin and for differentiation of paternally related males.

The results show that semenogelin and the staining degree of the aged sperm heads were not significantly affected. DNA degradation, assessed by RT-PCR, multiplex autosomal STR amplification and sequence analysis, affected the typing results according to the amplicon size. Therefore, the best choice for identification purpose in aged seminal stains can be assessed.

1. Connery SA. Three decade old cold case murder solved with evidence from a sexual assault kit: J Forensic Leg Med. 2013; 20: 355-336

225. COMPARISON OF DNA PREPARATION RESULTS FROM AUTOPSY TISSUE SPECIMENS WITH OR WITHOUT PREVIOUS POSTMORTAL CT ANGIOGRAPHY

Schwerer M. J.[1], Boehmer J., Graw M.[1], Fischer F.[1]

[1] *Institute of Forensic Medicine, Ludwig Maximilian University, Munich, Germany*

Michael.Schwerer@med.uni-muenchen.de

Background: Lipophilic contrast medium is administered through the vascular system during postmortal CT angiography. Following CT scan and forensic autopsy, the histological and molecular pathological assessment of lesions depends on tissue samples following chemical fixation. As a fixative, 4% to 10% buffered formalin solution is commonly employed, a substance known to aggravate DNA preparation. This study investigated if the presence of lipophilic contrast medium in tissues further impairs DNA extraction from autopsy exhibits. Material and Methods: Specimens from the heart of 15 autopsies following postmortal CT angiography were studied. For a comparison, 15 corresponding cases matched for a.) the pathological conditions in the myocardium, b.) the age and gender of the deceased, c.) the time between death and autopsy, and d.) the period of tissue fixation in formalin solution

between autopsy and DNA preparation were selected. A silica column based extraction system specially designed for formalin fixed tissues (Qiagen, Hilden, Germany) was applied. The effectiveness of DNA recovery was evaluated using a fluorescence based quantification technique (Life Technologies, Darmstadt, Germany). Results: Successful DNA preparations, albeit with low levels of DNA recovery, were seen in all specimens, irrespective whether the individual tissues were previously perfused with lipophilic contrast medium or not. Discussion: These preliminary data indicate that CT angiography does not further impair the already limited possibilities of molecular pathology on formalin fixed autopsy tissues. However, additional studies are necessary to define reliable procedures for molecular pathology on such material. Some examples on this will be presented and discussed.

226. VALIDATION OF AUTOMATED PCR SET-UP OF THE QUANTIFILER TRIO DNA QUANTIFICATION KIT ON THE BIOMEK 4000 LABORATORY AUTOMATION WORKSTATION

Janssen K., Olsen M., Olsen G.-H., Aune M., Berg T.

Forensic Genetics Centre, UiT The Arctic University of Norway, Tromsoe, Norway

kirstin.janssen@uit.no

The quantification of DNA in evidence samples is a necessary step to guide further processing of samples. Implementation of the new generation autosomal and Y-chromosomal STR-PCR kits has warranted the development of more accurate and informative DNA quantification kits. Hence, Life Technologies has recently released the Quantifiler Trio kit, which they claim has several improvements compared to previous quantification kits. In particular, they have addressed the challenges in determining the level of total DNA and male DNA in low-level samples. The kit also provides information on the level of degradation and inhibition. Automation of PCR-setups reduces the manual workload and the risk for human errors in forensic casework. We have validated the PCR-setup of the Quantifiler Trio kit on the Biomek 4000 (Beckman Coulter), a small benchtop automation workstation with a pod that uses single one-channel or multi-channel interchangeable tools. Particularly, we have tested the reproducibility and sensitivity of the kit, focusing on the human and male target. Furthermore, we conducted a mixture study with different male-female ratios. These mixtures were then amplified with the NGM Select PCR Amplification kit in order to correlate the quantification results to resulting DNA-profiles of mixtures. In respect to automation, we have also examined the level of cross contamination during plate setup. Our results so far show that the Quantifiler Trio kit is well suited for automation on the Biomek 4000. PCR set-ups produce consistent and reliable quantification results, both for the human and male target. Neither sample mix-up nor cross-contamination were observed.

227. EFFICIENCY OF DNA EXTRACTION FROM URINE TEST STRIPS

Tsukada K.

Department of Forensic Medicine, Criminal Investigation Laboratory, Nagano Pref. Police H.Q. Japan

tuk-lab@mx1.avis.ne.jp

Abstract

Presumptive tests are significant not just in the laboratory, but at crime scenes. Due to the range of evidence left at crime scenes, a key issue for crime-solving is learning as much as possible from the early stages of the investigation. Requirements for a presumptive test reagent include low cost, high sensitivity, and ease of use. For these reasons, we explored the suitability of urine test strips for use in presumptive tests for potential blood. At ISALM 2014, we reported on the sensitivity of and the influence of temperature and humidity on five kinds of urine test strips.

The present study discusses the efficiency of DNA extraction from these urine

strips.

Materials and methods

We used six kinds of urine test strips and a cotton swab. Specimens were prepared by placing blood on the urine test strips and cotton swab. Following specimen preparation, we extracted DNA using EZ1 Advanced XL immediately and one hour later. We performed PCR amplification using AmpFlSTR Identifiler Plus kit and electrophoresis using 3130xl Genetic Analyzer.

Results

We obtained full DNA profiles from three urine test strips and from the cotton swab. However, for one kind of strip, DNA typing was successful when the DNA was extracted immediately after specimen preparation, but we were unable to obtain the full DNA profile when DNA was extracted one hour later after specimen preparation. For two kinds of strips, we were unable to obtain the full DNA profile whether DNA extraction was performed immediately or one hour later.

228. DIRECT AMPLIFICATION OF REFERENCE SAMPLES WITH GLOBALFILER PCR AMPLIFICATION KIT

Gouveia N.[1,2], Brito P.[1,3], Serra A.[1], Balsa F.[1,3], Andrade L.[1,3], São Bento M.[1,3], Cunha P.[1], Bogas V.[1,3], Lopes V.[1,3], Porto M. J.[1,3]

[1] Forensics Genetic and Biology Service, Central Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal
[2] Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[3] CENCIFOR, Forensic Sciences Centre, Coimbra, Portugal

mariajoao.porto@dcinml.mj.pt

The GlobalFiler® PCR Amplification kit uses 6-dye fluorescent chemistry to enable multiplexing of 21 autosomal STRs, 1 Y-STR, 1 Y-indel and the sex-determining marker amelogenin. It is divided into two separate kits: GlobalFiler® Express that is a kit specifically designed for processing reference DNA samples and GlobalFiler® which is optimized for use with the most challenging casework samples. Validation studies were conducted to assess the performance of the GlobalFiler® casework kit for typing reference samples through a direct amplification. Blood and buccal DNA samples were collected on FTA® and FTA® indicating cards, respectively, and were allowed to dry at room temperature before using. For the validation study, 0.5, 0.75, 1 and 1.2 mm punches of each sample were added into the respective tube in a reaction volume of 12,5 µL. Thermal cycling was performed on a Veriti® 96-Well, followed by capillary electrophoresis on the 3500 Genetic Analyzer. HID files generated were analyzed using GeneMapper® ID-X Software v1.4. The results demonstrated that full and balanced profiles can be obtained from reference DNA samples using a 0.5 mm punch with the GlobalFiler® PCR Amplification kit.

References

[1] S. Flores *et al.*: Internal validation of the GlobalFiler™ Express PCR Amplification Kit for the direct amplification of reference DNA samples on a high-throughput automated workflow. Forensic Science International: Genetics. 2014; 10: 33–39.

[2] P. Martín *et al.*: Improving DNA data exchange: Validation studies on a single 6 dye STR kit with 24 loci. Forensic Science International: Genetics. 2014; 13: 68 –78.

[3] Applied Biosystems (2014). GlobalFiler® PCR Amplification Kit User Guide. Life Technologies.

229. DIRECT AMPLIFICATION OF CASEWORK SAMPLES IN FABRICS USING GLOBALFILER PCR AMPLIFICATION KIT

Brito P.[1,3], Gouveia N.[1,2], Bogas V.[1,3], Serra A.[1], Balsa F.[1,3], Andrade L.[1,3], São Bento M.[1,3], Cunha P.[1], Lopes V.[1,3], Porto M. J.[1,3]

[1] Forensics Genetic and Biology Service, Central Delegation,

National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal
[2] Faculty of Sciences, University of Lisbon, Lisbon, Portugal
[3] CENCIFOR, Forensic Sciences Centre, Coimbra, Portugal

mariajoao.porto@dcinml.mj.pt

After a validation study developed in the Forensics Genetic and Biology Service of Coimbra, Portugal, we were able to demonstrate the ability of the GlobalFiler® PCR Amplification kit in direct amplification of reference samples with consistent and reproducible results. Since this kit is optimized for casework samples, we decided to test it on forensic samples, in particular on fabrics stains. The selected samples had already been processed according to the methodologies normally used for this type of samples included automated extraction, quantification and amplification, followed by capillary electrophoresis, resulting in complete profiles. To develop our study, samples were collected from regions of the fabrics that had previously shown/proven to have biological evidence. The chosen samples were directly amplified for the GlobalFiler® PCR Amplification kit, followed by capillary electrophoresis on the ABI PRISM® 3500 Genetic Analyzer. The results obtained with this methodology revealed full and well balanced genetic profiles. This study intends to show that in some cases this approach can be an alternative, when we are in the presence of biological stains on fabrics, that allows to obtain consistent results in a short period of time.

[1] Applied Biosystems (2014). GlobalFiler® PCR Amplification Kit User Guide. Life Technologies.

230. TESTING THE BEHAVIOR OF GLOBALFILER PCR AMPLIFICATION KIT WITH DEGRADED AND /OR INHIBITED BIOLOGICAL SAMPLES

Bogas V.[1, 3, 4], Carvalho M.[2, 3], Corte Real F.[3, 4, 5], Porto M. J.[1, 2, 3, 4]

[1] Forensic Genetic Service, Centre Branch, National Institute of Legal Medicine and Forensic Sciences, IP, Coimbra – Portugal
[2] Forensic Genetic Service, South Branch, National Institute of Legal Medicine and Forensic Sciences, IP, Lisboa – Portugal
[3] CENCIFOR, Forensic Sciences Centre, Portugal
[4] Centre Branch, National Institute of Legal Medicine and Forensic Sciences, Coimbra – Portugal
[5] Medicine Faculty of the University of Coimbra – Portugal

mariajoao.porto@dcinml.mj.pt

The achievement of a complete genetic profile of degraded and/or inhibited biological samples is a challenging task for forensic scientists.

Our aim was to find out the behavior of GlobalFiler® PCR amplification kit in the presence of difficult samples and its ability to substitute the AmpFℓSTR® MiniFiler™ kit.

Bloodstains in denim, cotton and lycra were placed in different environmental conditions, during summer and winter seasons, for a maximum period of 2 years.

DNA extraction was performed using Chelex method, QIAamp® DNA Investigator kit and DNA IQ™ System kit. DNA was quantified with an ABI Prism®7000 using Quantifiler™ Human DNA Quantification kit or ABI Prism®7500 using Quantifiler™ Trio DNA Quantification kit. Samples were amplified with AmpFℓSTR® IdentiFiler™, AmpFℓSTR® MiniFiler™ and/or GlobalFiler® PCR Amplification kits, according to their quantification, and analyzed in ABI Prism®3130 and/or ABI Prism®3500 Analyzers.

In almost all analyzed samples GlobalFiler® kit enabled a complete genetic profile even when the sample had a DNA concentration as low as 0.01ng/ml, while in some of these samples Minifiler™ provided a partial profile or didn't enable any amplification.

1. Life Technologies, AmpFℓSTR® MiniFiler™ PCR Amplification Kit User's Guide, 2012
2. Life Technologies, GlobalFiler® PCR Amplification Kit User's Guide, 2012

231. DNA QUANTIFICATION BY REAL-TIME PCR IN DIFFERENT FORENSIC SAMPLES

Martins C.[1], Lima G.[2], Carvalho M. R.[3], Cainé L.[1,2], Porto M. J.[2]

[1] Department of Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Porto, Portugal
[2] National Institute of Legal Medicine and Forensic Sciences, North Department, Oporto, Portugal

mariajoao.porto@dcinml.mj.pt

The quantification of human DNA is an important procedure because it enables to ensure the competence of the assays carried out in forensic samples. The clear estimation about the quantity of DNA molecules, in order to overcome limiting factors, jointly enables the prevention of dissipation DNA sample used in assays. Its realization by Real-Time PCR appears to be an attractive assay for absolute quantification of unknown samples.

In the present study, the quantification of the human DNA was performed in three different types of biological samples (blood, buccal swabs and hair). *TaqMan® Universal PCR Master Mix* assay, was used with a previously described protocol for mitochondrial DNA (mtDNA). Nuclear DNA (ncDNA) was also quantified using *Quantifiler® Trio DNA Quantification Kit*. After DNA quantification, the samples were analyzed with different protocols to test their sensibility.

DNA quantification (human and mitochondrial) is an essential tool because it allows the forensic expert to decide what kind of DNA analysis is going to perform.

1. Niederstätter H, Köchl S, Grubwieser P, Pavlic M, Steinlechner M and Parson W: A modular real-time PCR concept for determining the quantity and quality of human nuclear and mitochondrial DNA. Forensic Science International. 2007; Volume: Genetics 1 page 29 – page 34.
2. Alonso A, Martín P, Albarrán C, García P, García O, Simón LF, García-Hirschfeld J, Sancho M, Rúa C and Fernández-Piqueras J: Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. Forensic Science International. 2004; Volume: 139 page 141 – page 149
3. Butler JM: Forensic DNA Typing – Biology, Technology, and Genetics of STR Markers. Elsevier Academic Press; 2005: page 33 – page 412.

232. DEVELOPMENT AND VALIDATION OF A HUMAN DNA QUANTIFICATION AND SEX DETERMINATION APPROACH BASED ON REAL TIME PCR FOLLOWED BY HIGH RESOLUTION MELTING ANALYSIS

Ginart S.[1]; Alechine E.[1], Caputo M.[1,2], Cano H.[3], Corach D.[1,2], Sala A.[1,2]

[1] Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry Universidad de Buenos Aires, Argentina
[2] Consejo Nacional de Investigaciones Científicas y Tecnológicas-CONICET, Argentina
[3] Laboratorio Regional de Investigación Forense, Poder Judicial de Santa Cruz, Argentina

dcorach@ffyb.uba.ar

DNA quantitation is one of the most crucial factors affecting the success and quality of DNA typing by PCR. The aim of this work was to develop a DNA quantification assay to be used in routine forensic casework. It should be able to discriminate, simultaneously, the presence of male and female DNA by means of multiplex real time PCR, followed by high resolution melting analysis (HRM), including a fluorescent intercalating dye Syto 9 due to its high sensitivity.

The approach consists in the co-amplification of gene fragments common to both genders, Amelogenin –Amel. The 6 bases pairs (bp) difference present in the sequences located at X chromosome and the Y counterpart generates a two peak pattern differing in 0.2°C. Aiming to boost the discrimination efficiency a human specific Y chromosome sequence (HSYCS) was also included in the reaction cocktail. The melting temperatures of this amplicon differ from Amel in

5.3-5.5°C. Hence, it allows discriminating two peaks after HRM analysis if only male DNA is present in the sample or a single peak if only female template is present. The short length of both amplicons, 106/112 bp for Amel and 84 bp for HSYCS, facilitates quantitation and gender detection in samples potentially degraded that characterize evidentiary material. We achieved the quantification of male, female and mixture samples with very low DNA quantities (20 pg/ul)

We propose an alternative approach to commercial DNA quantitation kits. Our development showed to be fast, highly sensitive, laborsaving and cost-effective.

233. VALIDATION OF A MULTIPLEX SYSTEM WITH 20 MULTI-INDELS FOR FORENSIC PURPOSES

Song F., Luo H., Li J., Hou Y.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, China

profhou@yahoo.com, forensic@scu.edu.cn

Multi-Indel polymorphism has been considered as a useful supplementary tool for human identification. Compared with the conventional single Indel marker, it has showed obvious advantage in improving the discrimination power. Previously, we established a novel multiplex system with 20 multi-Indels witch contains 43 single InDel markers. Currently, validation studies were performed with species specificity, sensitivity, mixture study and artificially degraded samples, according to the FBI/ SWGDAM guideline. The results showed (a) no fluorescent signal for forensically relevant animals was obtained, (b) complete and accurate profiles were detectable as DNA amount was 0.3 ng, and (c) the reliable results of typing 20 multi-Indels could be obtained from degraded DNA. The mixture of two unrelated individuals in a ratio up to 3:7 could be recognized with all alleles. In conclusion, this study demonstrated that the multiplex system with 20 multi-Indels can be used as a useful supplementary method for human identification and paternity testing.

234. AUTOMATING PREPFILER FORENSIC DNA EXTRACTION KIT: OPTIMIZATION AND VALIDATION ON FREEDOM EVO 150

Kallupurackal V.[1], Kausel T.[1], Sulzer A.[1], Voegeli P.[1], Kratzer A.[1]

[1] Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

venus.kallupurackal@irm.uzh.ch

To handle the growing number of forensic casework samples we decided to automate our trace sample workflow using PrepFiler®, a magnetic bead based DNA extraction kit, on a Tecan liquid handling workstation Freedom EVO® 150. Even though there was a manufacturer-supplied validated protocol for the automated PrepFiler® DNA extraction, substantial changes were needed in order to obtain the optimal DNA yield and elution volume to meet the needs of our laboratory for further downstream applications. In this study, optimal lysis condition, elution volume and elution temperature were determined for the automated PrepFiler® extraction using blood samples on cotton swabs. Additionally, a comparison with the manual in- house method Chelex-100® was made using a variety of mock forensic samples on swabs. We tested blood, saliva and contact traces from a variety of substrates, with 10 replicates of each type. With the automated PrepFiler® system, the DNA yield obtained was around 30-70% lower than with Chelex-100®. However, the purity of the extracts was higher. Allele recovery of the STR profiles obtained with AmpFLSTR® NGM SElect™ kit was comparable. Furthermore we found that, besides the kit-specific optimization factors, swab characteristics are of major importance for the success of PrepFiler® DNA extraction. We incorporated the optimized PrepFiler® protocol on Freedom EVO® 150 into our routine workflow and included quality controls to maintain contamination-free and efficient DNA extraction with no evidence for loss of magnetic beads.

235. NOVEL FORENSIC DNA PROFILING METRICS FOR A UNIVERSAL QUANTITATIVE VALIDATION SCHEME

Bessekri M. W.[1], Lazreg S.[1], Bucht R.[1,2], Fuller V. M.[2]

[1] Department of Biology - Institut National de Criminalistique et de Criminologie - Gendarmerie Nationale – Algeria
[2] US Department of Justice, International Criminal Investigative Training Assistance Program - Algeria

mouna1013@hotmail.com

Forensic DNA workflow requires a continuous effort from individual laboratories in order to combine reliability of results, minimisation of costs and convenience of the methods in use, all in line with the international recommended standards and interpretation guidelines. In this line of thought, an easy and efficient two-step validation study has been designed and conducted in our laboratory.

First, it was essential to assess the measurement of uncertainties for the main technologies used in the DNA analytical process. The parameters studied evaluated the x- and y-axis precision of the genetic analyzer and the limitations of the amplification kit in use, by measuring three objective metrics: 1) linearity 2) sensitivity and 3) empirically determined thresholds (including the analytical, the stochastic and the peak height ratio imbalance thresholds).

This valuable awareness led to the second step of the study, where we demonstrated that optimised analytical strategies can be adapted in our workflow based on the knowledge of the said parameters. We proved thus: 1) the value of reduced amplification volume valorising the amount of sample and reagents used 2) the merit of consensus profiling for low-template sample interpretation and 3) the benefits of introducing a novel, rapid scheme called "quantitative DNA profiling" which capitalizes on the quantitative data produced by the genetic analyzer.

In the prospect of implementing new techniques and processes, this study demonstrates the value of including these three objective metrics in the validation and optimisation of the DNA analysis process.

236. INVESTIGATION INTO ETHYLENE OXIDE RESIDUALS ON DOWNSTREAM DNA ANALYSIS

Yakovchyts D.[1], Wilson-Wilde L.[2], Neville S.[3], Maynard P.[1], Roux C.[1], Gunn P.[1]

[1] Centre for Forensic Science, University of technology Sydney, Sydney, Australia
[2] Australia New Zealand Policing Advisory Agency National Institute of Forensic Science, Melbourne, Australia
[3] Forensic and Analytical Science Service, NSW Health, Sydney, Australia

peter.gunn@uts.edu.au

Many decontamination techniques are used today in forensic laboratories. However, the preferred effective technique at reducing or eliminating endogenous DNA from case work swabs and laboratory consumables is ethylene oxide (EO) treatment. The aims of this study were to confirm the effectiveness of EO treatment and then to determine residual EO levels on swabs at various times after treatment, testing the effects of residual EO on crime scene DNA specimens .

The effectiveness of the EO treatment was tested by spiking cotton and rayon swabs with whole cells and analysing the DNA remaining on the swabs after the EO treatment. The effect of EO on downstream DNA analysis was tested by EO treating sets of cotton and rayon swabs and allowing 1, 7 or 21 days of storage of the swabs before using them to collect a specimen. Using commercial forensic STR kits, DNA analysis was performed at specific intervals (7, 14 and 35 days) and the results compared to control swabs.

The results showed that an adjusted 10 hour EO exposure is very effective at removing endogenous DNA from sealed swabs. The residual EO on swabs following treatment did affect downstream DNA analysis after 21 days of swab storage. The effects of residual EO were minimal in rayon swabs and more pronounced on cotton swabs, potentially hampered by inhibition effects of wooden swab stems. This should be considered in laboratory protocols for use of swabs.

237. VALIDATION OF THE AMPFLSTR NGM SELECT PCR AMPLIFICATION KIT USING ABI PRISM 310 GENETIC ANALYZER

Cerri N.[1], Cortellini V.[1], Verzeletti A.[1], Cisana S.[1], Agostino A.[2], De Ferrari F.[1]

[1] University of Brescia – Department of Medical and Surgical Specialties, Radiological Sciences and Public Health - Forensic Medicine Unit - Brescia, Italy
[2] Life Sciences Solutions, Thermo Fisher Scientific, Monza, Italy

nicoletta.cerri@spedalicivili.brescia.it

The AmpFlSTR® NGM SElect™ PCR Amplification Kit is a highly robust 17-plex STR genotyping kit developed for human identification laboratories wishing to add the highly polymorphic SE33 locus in order to expand European Standard Set of Loci. This kit shares the same primer sets for 16 common loci with the AmpFlSTR® NGM™ Kit (D10S1248, D3S1358, vWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, D22S1045, D2S441, D1S1656 and D12S391), with additional primers for the SE33 locus, delivering high sensitivity and improving STR performance for forensic casework and database samples in one easy workflow thanks to greater discriminatory power.

The AmpFlSTR® NGM SElect™ User Guide describes instrument setup and sample preparation of PCR products using the ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems 3130, 3130xl, 3500 and 3500xL Genetic Analyzers. In this study, we report the successful analysis of amplicons obtained with AmpFlSTR® NGM SElect™ on the ABI PRISM® 310 Genetic Analyzer, sequencer still widely used in many forensic laboratories. We performed developmental validation studies following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines, testing several critical areas of kit performance such as sensitivity, DNA mixtures and inhibited samples.

1. Life Technologies Corporation: AmpFlSTR® NGM SElect™ PCR Amplification Kit User Guide. Carlsbad, CA, USA. 2012. Available at: http://tools.invitrogen.com/content/sfs/manuals/cms_089008.pdf.
2. Scientific Working Group on DNA Analysis Methods: Validation Guidelines for DNA Analysis Methods. Approved at the Scientific Working Group on DNA Analysis Methods Meeting, Fredericksburg, Virginia, USA. December 2012. Available at: http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf
3. Green RL, Lagace RE, Oldroyd NJ, Hennessy LK, Mulero JJ: Developmental validation of the AmpFlSTR® NGM SElect™ PCR Amplification Kit: A next-generation STR multiplex with the SE33 locus. Forensic Sci Int Genet. 2013; 7:41–51.

238. A 21-PLEX DIP PANEL'S APPLICATION IN MULTINATIONAL CHINESE POPULATION

Jiang Y.[1], Wang L.[1], Li Z.[1], Peng D.[1], Zhu J.[1], Su Q.[1], Mao J.[2], Wang H.[2], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China

liangweibo@scu.edu.cn

Insertion/deletion polymorphisms (DIP) combining the advantages of both SNP and STR shows a great value in forensic case works. A high sensitive 21 AIM DIPs panel within 200bps developed by Daniel et al is sufficient to distinguish between three major global population groups (Europe, sub-Saharan Africa and East and South East Asia). However, Chinese population was not included in Daniel's research. In this study, DNA samples from three Chinese population groups living in west of China (Han (n=50), Uighur (n=30) and Tibetan (n=30)) were extracted and analyzed following Daniel's procedure. The difference in biogeographic ancestry between the Chinese Han population and the three major global population groups mentioned by Daniel was compared. Chinese internal difference was also researched. The consequence shows that Han

nationality differs from Europe and sub-Saharan Africa significantly. Difference between Chinese population and the East and South East Asian is trivial. Over this study, we observed that the flanking sequences of the 21 markers still have some other DIPs, which might lead to subtle variance. In summary, this 21-plex DIP panel is capable to distinguish the Chinese Han population from European and sub-Saharan African. To characterize three groups in China, more DIPs are needed to be added to the panel. The variation caused by DIPs in flanking sequence should be noticed. Furthermore, through sequencing technology platform, binding DIP with SNP and STR will be promising.

239. SCREENING OF MULTI-INDELS MARKERS ON X-CHROMOSOME FOR FORENSIC PURPOSE

Fan G.Y., Ye Y., Luo H., Hou Y.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

profhou@yahoo.com, forensic@scu.edu.cn

Many studies have been proposed to identify insertion/deletion (InDel) polymorphisms in humans for forensic genetics. However, the discriminatory power of InDel was limited by the poor polymorphism of diallelic marker. To improve discriminatory power, we designed the Multi-InDel comprising more than two InDel loci that were tightly linked by their physical position as a new marker, which can be amplified by a pair of PCR primers. This strategy gave at least three haplotypes for a multi-InDel marker. As genetic markers on X-chromosome have been recognized as useful tools to supplement traditional kinship testing, we focused on developing multi-InDel markers on the X-chromosome (X-Multi-InDel). We explored potential X-Multi-InDel from 1000 genome database, 10 candidates for X-Multi-InDel were selected. The frequencies of the haplotypes were also investigated in Chinese population. The results showed that there were higher levels of heterozygosity in X-Multi-InDel than X-SNP or X-InDel. It implied that X-Multi-InDel markers were useful for individual identification and relationship studies.

Theme 09: DNA Databases

240. BOOKING STATION DNA ANALYSIS AND SEARCHING OF CODIS DATABASES

Callaghan T.[1], Gerst J.[2], Wendel J.[1]

[1] FBI Laboratory Division, Quantico, Virginia, USA
[2] FBI Criminal Justice Information Services Division, Clarksburg. West Virginia, USA

Thomas.Callaghan@ic.fbi.gov

Commercial Rapid DNA (RDNA) instruments have been available for two years. These platforms were developed for automated typing and STR allele calling of buccal swabs for database enrollment and searching. Results are produced in approximately 90 minutes with minimal operator intervention. The presentation will discuss the FBI's two phase plan for booking suite integration of RDNA instruments into the United States National DNA Database. Electronic linking of arrestee information to the buccal swab for database enrollment will be discussed. Enhancements to CODIS for searching and hit notification to the booking station and investigating agency will also be presented. The concept of prioritizing the searching of "crimes of special concern" for immediate lights out hit notification will be introduced. With CODIS in use by more than 50 countries, arrestee enrollment enhancements have considered possible use by other countries. The use of an Electronic Biometric Transaction Standard (EBTS) with fingerprint based arrest records may allow for booking station adaptation by other CODIS counties that use electronic booking suite fingerprint enrollment systems.

241. CONSORTIUM VALIDATION PROJECT DATA ASSESSMENT TO FINALIZE THE EXPANDED CODIS CORE LOCI IN THE U.S.

Steffen (Hill) C. R.[1], Kline M. C.[1], Duewer D. L.[1], Vallone P. M.[1], Hares D.[2]

[1] National Institute of Standards and Technology, Gaithersburg, MD, United States
[2] FBI Laboratory, Quantico, VA, United States

becky.hill@nist.gov

In 2012 the FBI proposed to expand the number of CODIS core loci in the United States [1,2]. With the development of commercial STR multiplex kits containing the expanded loci, a consortium of 11 CODIS laboratories were selected to evaluate the performance of these kits in a collaborative validation study [3]. The study examined data from a variety of sample types including single source known, non-probative, challenge, and mixed samples. The study assessed concordance, precision, reproducibility, sensitivity, and mixture detection. The results were compiled and summarized using software developed at NIST to assist in the selection and adoption of the expanded CODIS core loci in the U.S. The presentation will explore a number of aspects of the data analysis including stutter and heterozygote balance results within the kits and lessons learned regarding the markers that led to final core loci. This presentation, in conjunction with "Implementation of the Expanded CODIS Core Loci in the United States" by Douglas Hares, will document the data driven decisions made to expand the CODIS core Loci in the U.S.

1. Hares D.: Expanding the CODIS core loci in the United States. FSI Genet. 2012; 6: e52-e54
2. Hares D.: Addendum to expanding the CODIS Core Loci in the United States, FSI Genet. 2012; 6: e135
3. Hares D.: Selection and Implementation of Expanded CODIS Core Loci in the United States. FSI Genet. 2015; 17: 33-34

242. THE USE OF DNA DATABASE OF BIOLOGICAL EVIDENCE FROM SEXUAL ASSAULTS AND RAPES IN CRIMINAL INVESTIGATIONS: A SUCCESSFUL EXPERIENCE IN BRAZIL

Ferreira S. T. G.[1,2], Paula K. A.[1], Maia F. A.[1], Svidzinski A. E.[1], Amaral M. R.[1], Diniz S. A.[1], Siqueira M. E.[1], Moraes A. V.[1]

[1] Forensic DNA Research Institute, Federal District Civil Police, Brasília, Brazil
[2] National Secretariat of Public Security, Ministry of Justice, Brasília, Brazil

samueldoc83@yahoo.com.br

DNA databases may play a crucial role in many kinds of criminal investigations. In sexual assaults, DNA databases of biological evidence may play an essential role in investigations and in the identification of the perpetrators. Particularly, in countries where there is no legislation regarding DNA databases from suspects or convicted individuals, DNA databases of biological evidence from sexual assaults and rapes may prove decisive in criminal investigations. In Brazil, until 2012 and 2013 there was no legislation concerning DNA databases from suspects or convicted individuals. As there was no legislation regarding this subject and due to the need for solving crimes related to sexual assaults, the Forensic DNA Research Institute of Federal District Civil Police, in Brasília, created in 1998 a DNA database of biological evidence from sexual assaults and rapes to aid criminal investigations. The aim of this study is to demonstrate the successful experience of the Forensic DNA Research Institute of Federal District Civil Police, in Brasília, Brazil, in aiding criminal investigations in solving crimes and in identifying perpetrators using this DNA database. Forensic scientists using a DNA database of biological evidence from sexual assaults working together with Federal District Civil Police authorities and investigators found 75 serial rapists who had attacked 210 women in Brasília, Brazil, until March, 2015.

243. PERFORMANCE TESTING OF THE SEMI-AUTOMATED CPA200 AND FULLY-AUTOMATED CPA300 CARD PROCESSING INSTRUMENTS

Skancke S.[1], Winitz S.[1], Savoldi G.[2], Gervasoni A.[2], Carbonaro A.[1]

[1] *Thermo Fisher Scientific, Human Identification Business, Oyster Point, CA, USA*
[2] *Copan Italia, R&D Brescia Italy*

Laboratories processing single-source reference samples are continuously looking to increase throughput and minimize sample backlogs. Thermo Fisher Scientific is the global exclusive distributor for the forensic market of the Card Processing Automation (CPA) systems developed by Copan to facilitate reference sample processing. Semi-automated systems (CPA200™ instrument) and fully-automated systems with liquid handling capabilities (CPA300™ instrument) are available. The two instruments are equipped with a variety of features to optimize punching performance, ensure sample integrity, and improve workflow efficiency, and can be integrated with a laboratory LIMS system. When paired with the NUCLEIC-CARD™ collection system and the GlobalFiler® Express PCR Amplification Kit or other Applied Biosystems® direct PCR amplification kits, the CPA200™ and CPA300™ instruments represent a complete solution to help laboratories meet the increasing challenges of developing or expanding a forensic DNA database.

The performance of CPA200™ and CPA300™ instruments was evaluated using the GlobalFiler® Express PCR Amplification Kit. Plate layouts were designed to test for cross-contamination, by organizing samples in zebra (alternating columns) and checkerboard (alternating wells) patterns. A CPA200™ instrument was used to punch saliva samples on NUCLEIC-CARD™ cards, FTA® Classic cards, and Bokumbio cards. A CPA300™ instrument was used to punch, dispense reagents, and seal plates of blood samples on NUCLEIC-CARD™ cards and saliva samples on NUCELIC-CARD™ cards and EasiCollect™ cards.

Cross-contamination was not observed in blank samples when one cleaning punch was used following sample punching, and expected genotypes were obtained from all wells that received punches with DNA. Static effects were not observed during this testing: sample discs were consistently released to the bottom of the plate well, and remained there during reagent dispensing and plate sealing.

For Research, Forensic, or Paternity Use Only. Not for use in diagnostic procedures. GlobalFiler® Kits: For Forensic or Paternity Use Only.

244. ALGORYTHM ALIGEN: SIMPLE METHOD OF ENCRYPTION AND MATCHING WITH GENETIC PROFILES IN A SYSTEM OF STR IDENTIFICATION DATABASE

Iannacone G.[1]

[1] *Legal Medicine and Forensic Science Institute, Lima, Perú*

ggiannacone@yahoo.com

During the DNA identification process of 15000 missing persons in Peru between 1980 and 2000, we observed many cases of random matches due to the population genetic structure (founder effect, low gene flow between communities and inbreeding). Since 2002 we have been developing an algorythm in Excel (ALIGEN) with the aim of improving the match and identification in this genetic context. ALIGEN performs a meiosis simulation in two databases (family and missing) building haplotypes profiles (hap-file) in groups of 4 markers (for 20 markers 5 "G" groups) and whose value or weight is expressed as $\sum_{n=1}^{r} N_n \cdot X_n^2$, where N is the fixed position in the hap-file and X is the repeat of each n STR allele in the hap-file. Simultaneously, performs a parentage analysis using the model of allele Identity by descendent (IBD), with a threshold of 90% in case of full siblings and parent-child. It is contrasted with a matching matrix generated in relation to the possibility of sharing the same hap-file using

$$\left(Ln\left(\left(\prod_{n}^{p}\left[\left(\sum_{n}^{q}Y_n\right)+1\right]\right)\right)\right)$$ -1, where Y represents the n values of hap-files shared between two individuals. Additionally, with the aim of ensuring DNA information privacy and thinking of the future of genetic databasing, we encrypted each genetic profile using where G is the n and n+1 values of the sum of the hap-file´s considered in a specific set of STR markers. This model corresponds to a Hash encryptation model. Finally, we also validated ALIGEN to solve the identification of missing persons in Honduras in 2012.

1. Iannacone, GC. Use of Microsatellites markers to determine the paternity of Alpaca Hucaya Race.Universidad Nacional Agraria la Molina, Magister Scientiae These 2005.
2. Liu W, Weir B.S., Genotypic probabilities for pairs of inbred relatives, Phil. Trans. R. Soc. B (2005) 360, 1379-1385.
3. Iannacone GC, Parra R, Bermejo M, Rojas Y, Valencia C, Portugues L, Medina M, Vallejo AR, Prochanow A, "Peruvian genetic structure and their impact in the identification of Andean missing persons: A perspective from Ayacucho", (2011) Forensic Science International: Genetics Supplement Series Vol. 3, Issue 1, Pages e291-e292
4. Iannacone, GC. Peru Criminal Offender DNA database. 5th Asian Forensic Science Network. Annual Meeting and Symposium - Singapur. Noviembre (2013) http://www.asianforensic.net/documents/AFSN%20Programme_2013_for%20website.pdf

245. NEW ZEALAND'S DNA PROFILE DATABANK- CELEBRATING 20 YEARS OF SUCCESS.

Harbison S. A., Bright J. A.

Institute of Environmental Science and Research, ESR Mt Albert Science Centre, Private Bag 92-021, Mt Albert, Auckland, New Zealand

SallyAnn.Harbison@esr.cri.nz

In 1995 the Criminal Investigations (Blood Samples) Act (New Zealand) was passed into legislation. Since that time more than 156 000 profiles from individuals, comprising 3.5% of the total population, and 33,219 case sample profiles have been added to the DNA Profile Databank generating link rates of 29% (case sample to case sample) and 70% (case sample to person) respectively. Following the highly successful implementation of the DNA Profile Databank, our attention turned to maximising the effective use of the information held, accompanied by legislative changes which have been introduced to enable more effective use of the data and ensuring efficient and targeted collection of samples from individuals. Examples include using familial searching to assist in the solution of crime and mixture matching- the automated searching of unresolved mixed profiles from case samples. This poster summarises the successful technical operation and outcomes of the DNA Profile Databank demonstrating its effectiveness in crime solution in New Zealand and discusses options for future development in the light of advances in massively parallel sequencing approaches.

246. SMARTRANK: A LIKELIHOOD RATIO SOFTWARE FOR SEARCHING NATIONAL DNA DATABASES WITH COMPLEX DNA PROFILES

Haned H.[1], Gill P.[2,3], de Jong J.[1], van der Beek K.[1]

[1] Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands
[2] National Institute of Public Health, Department of Forensic Biology, P.O. Box 4404 Nydalen, 0403 Oslo, Norway
[3] University of Oslo, Oslo, Norway

h.haned@nfi.minvenj.nl

DNA profiles that are both incomplete and mixed are commonly encountered in forensic casework. Searching a DNA database with such profiles usually yields very large numbers of partial matches that can present many candidate suspects to be further investigated by the police. Current practice in most forensic laboratories consists of ordering these 'hits' based on the highest

number of matching alleles with the searched profile. However, this method does not allow differentiating hundreds if not thousands of candidate profiles that share similar numbers of matching alleles and is subject to both high false positive and false negative errors.

Ranking the hits based on a likelihood ratio (LR) score has been demonstrated to improve the representation of the strength of association between the candidates in the database and the searched profiles [1], however this was not extended to partial DNA profiles.

In this poster, we introduce the open-source SmartRank software: a user-friendly software that enables searches of complex profiles, based on LR scores. We assessed the efficiency of the SmartRank software using an anonymous copy of the Dutch DNA database and experimental mixtures with varying levels of partiality. Our results show that the program is very useful to find multiple candidates that can simultaneously explain complex DNA profiles, therefore generating useful intelligence data.

1. Y-K. Chung and W. K. Fung. The evidentiary values of "cold hits" in a DNA database search on two-person mixture. Science & Justice. 2011; 51(1):10-15.

Theme 10: Body Fluids Identification

247. A PROBABILISTIC APPROACH FOR THE INTERPRETATION OF RNA PROFILES AS CELL TYPE EVIDENCE

de Zoete J.[1], Sjerps M.[2], Curran J.[3], Maaskant P.[1]

[1] *University of Amsterdam, The Netherlands*
[2] *Netherlands Forensic Institute, The Hague, The Netherlands*
[3] *The University of Auckland, New Zealand*

DNA profiles can be used as evidence to distinguish between possible donors of a crime stain. In some cases, both the prosecution and the defence claim that the cell material was left by the suspect but they dispute which cell type was left behind. For example, in sexual offense cases the prosecution could claim that the sample contains semen cells where the defence argues that the sample contains skin cells. In these cases, traditional methods can be used to examine the cell type contained in the sample. However, there are some drawbacks, such as a small amount of questioned material, that may limit their application.

Another option is messenger RNA (mRNA) evidence. mRNA expression levels vary among cell types and can be used to make (probability) statements about the cell type(s) present in a sample.

Existing methods for the interpretation of RNA profiles as evidence for the presence of certain cell types aim for making categorical statements. Such statements limit the possibility to report the associated uncertainty. From a statistical point of view, a probabilistic approach is a preferable choice. In this talk we discuss two existing approaches, and propose two probabilistic methods. All methods are compared, using two different datasets and several criteria regarding their ability to assess the evidential value of RNA profiles.

248. ADVANCING FORENSIC RNA TYPING: NON-TARGET BODILY SECRETIONS, A NASAL MUCOSA MARKER, A DIFFERENTIAL EXTRACTION PROTOCOL AND THE RELATION BETWEEN DNA AND RNA PROFILING SENSITIVITY

van den Berge M.[1], Bhoelai B.[1], Harteveld J.[1], Matai A.[1], Sijen T.[1]

[1] *Netherlands Forensic Institute, The Hague, The Netherlands*

m.van.den.berge@nfi.minvenj.nl

The forensic identification of human body fluids and tissues by means of messenger RNA (mRNA) profiling is a long studied methodology that is increasingly applied to casework samples. Previously, we have described an mRNA multiplex system that targets blood, saliva, semen, menstrual secretion, vaginal mucosa and skin. In this study we consider various topics to improve this mRNA profiling system or its use, and adapt the method accordingly. Bodily secretions that may be encountered at a crime scene whilst not targeted by the multiplex –i.e. nasal mucosa, sweat, tears, faeces and urine- were examined for false positive signals. The results prompted us to identify a nasal mucosa marker that allows the discrimination of nasal mucosa from saliva or vaginal mucosa and nosebleed blood from peripheral blood. Lactobacillus markers were discarded as replacement for vaginal mucosa mRNA markers because of background signals on penile swabs that appeared devoid of female DNA. Furthermore, we explored an approach to deal with highly unbalanced mixtures by incorporating a differential extraction protocol into a co-extraction protocol to allow DNA and RNA analysis of separated non-sperm and sperm fractions. Lastly, we assessed the sensitivity of DNA or RNA profiling for different body fluids. While some sample types such as blood and semen show the trend that DNA profiling is more sensitive than RNA profiling, the reverse is seen for skin and variable results occur for vaginal and nasal mucosa. These results assist in furthering (forensic) RNA profiling.

249. PERFORMING BODY FLUID IDENTIFICATION USING A MICRORNA MULTIPLEX WITH CAPILLARY ELECTROPHORESIS

van der Meer D. J., MSc[1], Williams G., Dr[1]

[1] *School of Applied Sciences, University of Huddersfield, United Kingdom*

d.vandermeer@hud.ac.uk

MicroRNAs have a potential to be ideal forensic markers due to their small size (~22nt), high abundance per cell, and sensitive and specific PCR-based detection. Thousands of microRNAs are present in biological material and they are rich in information due to their tightly regulated expression, and can therefore be used for body fluid identification (BFID). Their advantageous properties increase the chances of successful analysis from challenged crime scene samples. In addition, it has been demonstrated that informative microRNA expression levels can be obtained from common DNA extracts. Following an earlier pilot project on a single stream process with the integration of microRNA analysis into a DNA profiling multiplex, progress on this line of research is now presented. A panel of 8 microRNAs (hsa-miR-10a, -16a, -135a, -142, -203a, -205, -451a and -1260b) has been identified to allow differentiation between blood, saliva, semen and vaginal material. Here the analysis of the endogenous control (SNORD44) and the BFID markers using capillary electrophoresis on ABI's 3130 genetic analyser is presented in single- and multiplex, and the effects of combining their analysis with genomic DNA human identification STR markers in a single reaction are explored. The markers are reverse transcribed using a multiplex stem-loop reverse transcription, followed by multiplex PCR with labelled primers for the cDNA and genomic DNA markers simultaneously. Future work will include the incorporation of the additional body fluid specific markers, working towards a single reaction that can provide a DNA profile and body fluid identification on single source and mixed samples.

250. NOVEL MESSENGER RNAS FOR BODY FLUID IDENTIFICATION

Albani P.[1,2], Patel J.[2], Fleming R.[2]

[1] School of Chemical Sciences, The University of Auckland, Auckland, New Zealand
[2] The Institute of Environmental Science and Research Ltd. (ESR), Private Bag 92021, Auckland, New Zealand

Patricia.Albani.Student@esr.cri.nz

In legal investigations it can be beneficial to not only know the donor of biological evidence, but also its cellular origin [1]. Conventional chemical and enzymatic tests are often insufficient in terms of specificity and sensitivity. Messenger RNA (mRNA) profiling based on unique gene expression patterns in cells and tissues can overcome these limitations and has become a valuable tool in forensic biology.

Some mRNAs presently used are controversial with respect to their body fluid specificity [2]. Therefore, the successful identification of samples may require detecting multiple mRNAs per body fluid simultaneously. The aim of this work is to characterise new mRNAs that can supplement existing markers for the detection of venous blood, semen, saliva, menstrual fluid and vaginal secretions.

Twenty six mRNA candidates, some identified from massively parallel sequencing data, have been evaluated for their respective expression patterns by reverse transcription polymerase chain reaction (RT-PCR). Eight of these are promising new markers that have shown to be specific and more sensitive for their respective target body fluids than previous mRNA markers. These novel mRNAs can be used as additional targets for improved body fluid profiling.

1. Fleming RI, Harbison S: *The development of a mRNA multiplex RT-PCR assay for the definitive identification of body fluids.* Forensic Science International: Genetics. 2010; 4:244-256
2. Van den Berge M, Carracedo A, Gomes I, et al.: *A collaborative European exercise on mRNA-based body fluid/skin typing and interpretation of DNA and RNA results.* Forensic Science International: Genetics. 2014; 10:40-48

251. BODY FLUID SPECIFIC MICRORNA MARKERS: CHARACTERISING THE FLUCTUATION OF EXPRESSION IN VAGINAL MATERIAL WHEN ANALYSED OVER A FULL MENSTRUAL CYCLE.

Bexon K.[1], Williams G.[2]

[1] School of Applied Sciences, The University of Huddersfield, Huddersfield, United Kingdom

Kimberley.Bexon@hud.ac.uk

The identification of body fluids (BF) is vital for progressing alleged sexual assault cases. The capability to do so however, is limited. This is due to the non-existence of presumptive BF identification tests for vaginal material and aspermatozoic seminal fluid. Equally, where blood is present, distinguishing between menstrual and venous blood is also problematic. The characterisation of microRNA: short, stable, non-coding RNA's which modulate gene expression post transcription present a solution to BF identification problems[1, 2]. Their precise role in gene regulation suggests the presence of BF specific microRNAs is likely, thus making them a robust tool for BF identification. Screening of numerous microRNA resulted in a set of markers capable of differentiating a full BF panel. The menstrual cycle consist of physiological changes such as ovum release and the building and detachment of the endometrial lining as well as the fluctuating hormonal changes of oestrogen, progesterone and FSH. These dynamic changes are likely to be regulated by microRNA and therefore their expression level may vary at throughout the cycle. Evidence of these microRNA expression fluctuations throughout the menstrual cycle is currently lacking within the literature. A full BF panel of markers were used to analyse daily vaginal material samples provided by 5 females over the course of 31 days. Expression of microRNA was measured using stem-loop reverse transcription, followed by qPCR. This study presents the first characterisation of microRNA

expression over a full menstrual cycle, demonstrating their robustness as a successful BF identification tool.

1. Bai, P., et al., *Micro RNA profiling for the detection and differentiation of body fluids in forensic stain analysis.* Forensic Science International: Genetics Supplement Series, 2013. 4(1): p. 216-217.
2. Park, J.L., et al., *Microarray screening and qRT-PCR evaluation of microRNA markers for forensic body fluid identification.* Electrophoresis, 2014.

252. MICRORNA MARKERS FOR FORENSIC BODY FLUID IDENTIFICATION OBTAINED FROM MIRCURY LNA ARRAY

Luo X.[a,1], Li Z.[a,1], Peng D.[1], He W.[1], Zhu W.[1], Wang L.[1], Wang H.[2], Mao J.[2], Liang W.[1], Zhang L.[1]

[1] College of life Sciences, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China
[a] These authors contributed equally to this work

liangweibo@scu.edu.cn

MicroRNA is a promising marker for forensic body identification especially in compromised samples and mixtures because of its small size and tissue specific expression manner. Previously, five research groups have reported specific miRNAs screened from different arrays for body fluid identification. Nonetheless, few of their results overlapped and repeated each other. Our study aims to screen appropriate miRNAs for forensic body fluid identification through Exiqon's miRCURY™ LNA Array annotated in miRBase 18.0. Five samples of six common kinds body fluids containing peripheral blood, menstrual blood, saliva, semen, vaginal secretion and skin cells were collected. Six miRNAs were selected as novel candidates for forensic body fluid identification: miR-190a-5p, miR-454-3p for peripheral blood; miR-141-3p, miR-497-5p for menstrual blood; miR-935, miR-3168 for saliva. Reliable miRNA markers for semen, vaginal secretion and skin cells couldn't be identified because relative expression ratios from array results were not large enough to identify each other. According to this, maybe more markers should be combined together to identify the three body fluids, and the strategy need to be refined. We also examined reported miRNAs of previous studies in our miRNA microarray dataset. While fifteen of them showed body fluid-specific expression patterns in our dataset, such as miR-135a-5p and miR-888-5p for semen; miR-451a, miR-144-3p for blood, but they were not the best candidates. Confirmation of new selected markers is underway. Our study first introduces skin cells into the microarray screening and enrich the candidates for forensic body fluid identification.

253. INFLUENCES OF DIFFERENT QPCR METHODS ON FORENSIC BODY FLUIDS IDENTIFICATION BY MICRORNA

Li Z.[a,1], Bai P.[a,1], Peng D.[1], He W.[1], Zhu W.[1], Wang L.[1], Wang H.[2], Long B.[3], Liang W.[1], Zhang L.[1,]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Institute of Forensic Science Chengdu Public Security Bureau, Chengdu 610081 Sichuan, China
[3] Department of Criminal Science and Technology, Sichuan Police College, Luzhou 646000, Sichuan, China
[a] These authors contributed equally to this work

liangweibo@scu.edu.cn

MicroRNA (miRNA) has attracted wide interest of the forensic community since Hanson attempted to search specific microRNA for body fluid identification from microarray. Research groups have screened out some sets of miRNA markers that can be used in forensic body fluids identification up to now. However, only several miRNA markers were confirmed by different groups. Most of those miRNA markers could not be repeated in different labs. The

methods of those labs are different, such as microarrays used to screen the markers and the qPCR methods. The markers confirmed by SYBRgreen always could not be confirmed by another group in Taqman. Different quantitative PCR methods might be the major cause of that. To seek out the influences of different quantitative PCR methods on the expression of miRNA, we chose thirteen miRNAs which showed conflicted or consistent results in different qPCR methods according to previous studies, including miR-451, miR-16, miR-10a, etc. for blood, semen, menstrual blood, saliva and vaginal secretions (ten for each). MiRNAs were analyzed through Taqman and SYBRgreen according to the protocols respectively. The amplicons were sequenced after being analyzed by agarose and polyacylamide gel electrophoresis. Different Ct values of the same miRNA markers were detected by SYBRgreen and Taqman, such as the expression of miR-658 for saliva was good in Sybgreen but little in Taqman, and the expression of miR-135b for semen was good in Taqman but couldn't be repeated in SYBRgreen . Our study has proved that the miRNA of the same sample may show different results in different qPCR methods.

254. SCREENING OF MICRORNA MARKERS FOR DISTINGUISHING BETWEEN MENSTRUAL AND PERIPHERAL BLOOD

Li Z.[a,1], Bai P.[a,1], Peng D.[1], He W.[1], Zhu W.[1], Wang L.[1], Mao J.[2], Long B.[3], Liang W.[1], Zhang L.[1]

[1] *Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China*
[2] *Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China*
[3] *Department of Criminal Science and Technology, Sichuan Police College, Luzhou 646000, Sichuan, China*
[a] *These authors contributed equally to this work*

liangweibo@scu.edu.cn

The identification of menstrual blood is an essential issue in forensic practice. Identifying the origin of the blood left at a crime scene is crucial for crime scene reconstruction, especially in sexual assault. Menstrual blood is composed of three distinct body fluids: blood, vaginal secretion and fluid of the late secretory phase of the uterine endometrial lining. However, there is no reliable and confirmatory assays for identification of menstrual blood. MiRNAs (MicroRNAs) are non-protein coding molecules with an important regulatory role at the post-transcriptional level. Since Hanson found some miRNA markers to identify the body fluids, lots of scientists have begun to study on the miRNA markers screened for body fluid identification. Now, three miRNA groups for menstrual blood identification have been identified. But none of them overlapped and has been approved each other. In our study, we attempt to screen the new markers for menstrual blood through Exiqon's miRCURY™ LNA Array annotated in miRBase 18.0. Four miRNAs were selected according to the result of the microarray: miR-141-3p, miR-497-5p, miR-143-5p, miR-136-5p whose expression levels are 27.95, 16.74, 10.14, 9.21 fold changes respectively comparing with peripheral blood, and confirmation of those markers is underway. According to the markers, a multi-steps procedure of blood identification was set up. First, blood should be recognized by blood-specific makers such as miR-451 and miR-16, then menstrual blood could be identified from peripheral blood by new markers, such as miR-141-3p, miR-497-5p, miR-143-5p and miR-136-5p.

255. VALIDATION OF FORENSIC BODY FLUID IDENTIFICATION BASED ON EMPIRICALLY NORMALIZED MICRO-RNA EXPRESSION DATA

Sauer E.[1], Madea B.[1], Courts C.[1]

[1] *Institute of Legal Medicine, University of Bonn, Bonn, Germany*

e.sauer@uni-bonn.de

Since 2003, mRNA analysis has been addressed in forensic research as alternative to the conventional methods for body fluid identification which exhibit disadvantages regarding specificity, sensitivity and sample

consumption. In recent years, the potential of forensic microRNA analysis was being assessed because of certain advantages over messenger RNA based methods, particularly the miRNAs' small size renders them less susceptible to degradation.

Based on an evidence based normalization strategy for quantitative PCR data from miRNA expression analysis in forensically relevant body fluids [1] we now present the validation of a panel of body fluid specific miRNA markers. Promising candidate microRNAs were selected by microarray profiling covering miRBASE v.18.0 and from the available forensic literature. The comprehensive validation study encompassed aged and challenged samples exposed to various conditions as well as mixtures emulating traces e.g. as typically encountered in sexual assault crimes. Based on our findings we aim to establish a robust mathematical model to predict in a binary fashion the presence or absence of a given body fluid in samples of unknown composition.

1. Sauer E, Madea B and Courts C: *An evidence based strategy for normalization of quantitative PCR data from miRNA expression analysis in forensically relevant body fluids.* Forensic Sci Int Genet. 2014; 11: 174-81

256. TISSUE-SPECIFIC-METHYLATION PATTERN ASSAY IN CASEWORK

Teschner J., Otremba P., Nagy M.

Department of Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité-Universitätsmedizin, Berlin, Germany

Jessica.Teschner@charite.de

In sexual cases, the most common source of biological material is semen. One of the standard detection methods of semen is the protein-based immunological assay. We established an alternative method which is based on DNA and uses a methylation sensitive restriction enzyme. These assays result in a tissue-specific-methylation pattern (TSMP) and allow the differentiation between semen and non-semen samples. In a primarily study we optimized an already published method [1, 2] in our laboratory and found a new semen-specific marker. This new marker was combined with the existing multiplex assay. Recently we analyzed in our routine work sexual cases additionally to the standard method RSID (Galantos) with the newly established TSMP assay to test the sensitivity and the performance in casework samples. One of the problems in casework samples is the mixture of body fluids and the different concentrations of these components. To overcome this problem we tried different dilutions of the samples to improve the detection of the minor component. A further problem in casework samples are the generally low DNA concentration. In order to achieve clear results, the reaction requires a highly optimized enzyme concentration. The present study will show the pros and cons of TSMP in casework and will give an outlook of the possibilities, which are given in combination with autosomal STRs.

1. Frumkin, D., et al., *DNA methylation-based forensic tissue identification*. Forensic Sci Int Genet, 2011. 5(5): p. 517-24.
2. Wasserstrom, A., et al., *Demonstration of DSI-semen--A novel DNA methylation-based forensic semen identification assay*. Forensic Sci Int Genet, 2013. 7(1): p. 136-42.

257. VALIDATION OF AN IMMUNOCHROMATOGRAPHIC D-DIMER TEST TO PRESUMPTIVELY IDENTIFY MENSTRUAL FLUID IN FORENSIC EXHIBITS

Holtkötter H., Dierig L., Schürenkamp M., Sibbing U., Pfeiffer H., Vennemann M.

Institute of Legal Medicine, University Clinic Münster, Germany

Hannah.Holtkoetter@ukmuenster.de

Identifying the biological source of a crime scene stain can be crucial for police investigations in many scenarios. Blood is one of the most common fluids found, and accurate differentiation between peripheral blood and menstrual

fluid could provide valuable information regarding the issue of consent in sexual assault cases. For the detection of menstrual fluid, no easy-to-use presumptive test is available to date. Thus, the aim of this study was to evaluate and validate a simple presumptive test for the indication of menstrual fluid in forensic stains, focusing on a simple immunochromatographic test that is based on the detection of degradation products of fibrinolysis, namely D-dimers. D-dimer testing was initially developed as diagnostic assay for e.g. thrombosis or embolisms. The use of D-dimers to identify menstrual blood has been initially described by Miyaishi and colleagues in 1996 (1) and the idea has been picked up recently by Baker et al. (2). We now report the first forensic validation of an immunochromatographic assay, the Clearview® rapid D-dimer test. We validated the sensitivity and robustness of the assay using fresh and dried menstrual fluid samples, body fluid mixtures, diluted samples, and casework swabs. Cross reactivity was tested for saliva, semen, vaginal fluid, and blood. No false positive results were obtained; it was possible to successfully analyze mixtures, highly diluted samples, and casework swabs. The results of this study indicate that the D-dimer assay reliably detects menstrual fluid in forensic exhibits and is easy to implement into the current workflow of body fluid identification.

1. Miyaishi S, Kitao T, Yamamoto Y, Ishizu H, Matsumoto T, Mizutani Y, Heinemann A, Püschel K: *Identification of menstrual blood by the simultaneous determination of FDP-D dimer and myoglobin contents*,1996, Nihon Hoigaku Zasshi, Volume: 50, Pages 400–403
2. Baker DJ, Grimes EA, Hopwood AJ: *D-dimer assays for the identification of menstrual blood*, 2011, Forensic Sci Int, Volume: 212, Pages 210–214

258. MICRORNA PROFILING FOR CHARACTERIZATION OF (MICRO) TRACES

Kulstein G.[1], Miltner E.[1], Wiegand P.[1]

[1] *Institute for Legal Medicine, University Ulm, Ulm, Germany*

Galina.Kulstein@uniklinik-ulm.de

In the last year the analysis of non-coding RNA, particularly microRNA (miRNA) has caught attention in the field of forensic body fluid identification. Several studies identified candidate markers – at least for blood and saliva – and showed their potential to assign blood and saliva traces.

Even though studies with identified markers and their suitability for e.g. degraded or minute traces are available, ample challenges and pitfalls still exist. Especially, the problems of normalization and the optimal processing of forensic samples for miRNA analysis are still unsolved. To this day, different approaches for isolation of miRNA as well as for downstream analysis of miRNA samples are used in distinct studies, thus requiring a standardization of miRNA profiling for forensic purposes. In this study, we investigate the efficiency of miRNA profiling and its adaptability into the forensic workflow of an established laboratory.

As a first step we evaluated miRNA isolation as well as miRNA and DNA co-extraction methods and compared automated versus manual protocols. The results show that combining an automated extraction method with specific high-sensitivity probes is a promising strategy for the identification of body fluid traces.

In the following analysis, the best ascertained strategy for miRNA and DNA co-extraction was applied to traces from recent cases – especially focusing on specimen that could not been analyzed with conventional techniques for body fluid identification due to interferences or method limitations. Herein we present the capability of miRNA profiling for challenging cases comprising buried (bloody) weapons and blood/bleach-mix soaked cloths.

259. CONFIRMATORY DETECTION OF SPERM VIA PROXIMITY LIGATION REAL TIME PCR

Riman S.[1], Shek C.[1], Podini D.[1]

[1] *Department of Forensic Sciences, GWU, 2100 Foxhall rd NW, Washington DC, US*

podini@gwu.edu

Currently microscopic examination of spermatozoa remains the sole method to confirm sperm in sexual assault evidence. The process is labor-intensive prompting the search for alternative approaches. This study demonstrates the potential use of Proximity Ligation Real Time PCR (PLiRT-PCR) as a novel forensic tool for microscope-free confirmatory detection of spermatozoa from sexual assault evidence. PLiRT-PCR was employed by using pairs of antibodies anti-Crisp2, an acrosome-specific protein, attached to unique DNA strands. When the pairs of antibodies bind to the target, the strands come into close proximity and bind to a complementary connecting oligo added to the solution. These oligos are then ligated forming a new DNA strand that is detected by real-time PCR. The quantity of the amplified DNA corresponds to the amount of protein target, which is proportional to the amount of sperm cells in the sample. In this study, a set of five serial dilutions of human semen was prepared ranging from 1:100 to 1:5000. Five 10 µL aliquots of the 1:1000 dilution were placed on a hemocytometer; an average of 30 sperm cells were counted in each aliquot. Same volume aliquots from each dilution were then placed in a new tube and centrifuged to pellet the sperm cells, which were then subjected to PLiRT-PCR in a 96-well plate on an ABI 7500. The assay detected sperm cells in all dilutions (approximately six sperm cells) demonstrating that PLiRT-PCR is sensitive, has high-throughput potential, minimizes sample consumption, and can overcome the drawbacks associated with conventional microscopic sperm search.

260. COMPARISON OF THREE CO-EXTRACTION KITS FOR TOTAL RNA AND GENOMIC DNA FROM MOUSE CARDIAC MUSCLE

Zhu W.[1], Jin B.[2], Wang L.[1], He W.[1], Li Z.[1], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Medicine, North Sichuan Medical College, Nanchong 637000, Sichuan, China

liangweibo@scu.edu.cn

Generally, a false positive result could be caused by genomic DNA contamination in highly sensitive experiment involved with RNA, thus the key point of such application is to isolate DNA-free RNA. There are always some exceptional circumstances, under which the synchronous extraction technique of RNA and DNA is necessary to interpret the potential practical advantage of RNA. In our previous research strategy on postmortem interval, we have used the genomic DNA, which was co-extracted with RNA, as reference genes for normalization during statistical analysis to study the relationship between the degradation of mRNA and postmortem interval. And the efficiency of reverse transcription has been tested before we used the DNA as a normalization gene, such as DAPDH, β-actin. Also the DNA and RNA co-extraction method plays very important role in the strategy. To established a most capable and stable technique for co-extracting total RNA and genomic DNA, we applied and evaluated three most popular used co-extraction techniques, the Allprep DNA/RNA/miRNA Universal Kit (QIAGEN), the Genome DNA and RNA Co-extraction Kit (Bio Teke) and Trizol, using myocardial tissue of mice with similar and two different weights (to investigate the minimum amount for effective extraction). Optical density and integrity of products were tested respectively through ultraviolet spectrophotometer, Qubit and qPCR. Results indicated that the Genome DNA and RNA Co-extraction Kit presented the best performance when co-extracting of total RNA and genomic DNA.

261. EVALUATION STUDY ABOUT THE SERATEC RAPID TESTS

Barbaro A.[1], Cormaci P.[1] , Votano S.[1] , La Marca A.[2]

Studio Indagini Mediche E Forensi (SIMEF) – ITALY
[1] Dept. Forensic Genetics - [2] Dept. Legal Medicine

simef_dna@tiscali.it

SERATEC® Immunochromatographic rapid tests are commonly used for confirming the presence of human blood, semen or saliva in a variety of forensic samples.

These tests use two monoclonal antibodies that form a sandwich complex with Hemoglobin, PSA or Amylase if they're present in the sample material. This complex can be seen on the membrane as the red test result line allowing a clear and easy interpretation of the test result Seratec provides in the kits a buffer which ensures maximum extraction efficiency

The aim of present study was to confirm the efficacy of the test to detect old samples even in presence of other biological fluids and especially to verify that the extraction medium do not have a negative effect on the DNA profile, so to ensure that routine use of Seratec tests would be compatible with laboratory DNA typing procedures.

Different forensic samples diluted in the buffer were tested in comparison with same samples eluted in water.

For DNA extraction 3 different procedures (rapid-resin, silica-column, magnetic beads) were used .

No significant differences were observed in the extraction efficiency, DNA recovery, and quality of DNA profiles obtained.

The use of these kits would provide a great contribution in the investigation by allowing good efficiency also in subsequent DNA analysis.

262. ADDITIONAL FIXATION IMPROVES THE PERFORMANCE OF SPERM HY-LITER EXPRESS

Olsen G.-H.[1], Aune M.[1], Janssen K.[1] , Berg T.[1]

[1] Institute for Forensic genetics, University of Tromsoe, The arctic university of Norway, Tromsoe, Norway

Gunn-Hege.Olsen@uit.no

The detection of spermatozoa in sexual assault evidence is important as it may confirm sexual activity and guide further processing of samples. The SPERM HY-LITER Express kit (Independend Forensic) is widely used by forensic laboratories since it stains human sperm cells specifically. The sperm cells are visualized by fluorescence microscopy.

During the internal validation of SPERM HY-LITER Express, we experienced that the fixation of cells to the slides represents a challenging step in the protocol. If the cells are not properly fixed to the slide, the cells may be lost during the subsequent staining and washing steps. In order to optimize fixation of cells, we have therefore tested slides from different manufacturers and different fixation strategies. For these experiments, cells were extracted from vaginal swabs and mixed with a known number of sperm cells. Aliquots of this stock were transferred to the sample windows of the slides and stained either according to the recommended protocol from the manufacturer or using a modified protocol with an additional fixation steps.

Preliminary results indicate that an additional fixation step increases the number of cells fixed to the slides. Irrespective of slide type, this is the case for both epithelial cells and more importantly sperm cells. Considering the impact the detection of sperm cells has on casework, improved sensitivity of SPERM HY-LITER Express is highly important.

263. LESS BRIGHT CF488 FLUORESCENCE SIGNALS IN NON-SPERMATIC NUCLEI

Martínez P.[1], Vallejo G.[1]

[1] National Institute of Toxicology & Forensic Science José Echegaray 4 -28232 Las Rozas de Madrid, Spain

pilarm.fernandez@justicia.es

The objective was to research challenged sexual aggression samples using fluorescent staining, particularly in the presence of yeast or highly refringent debris. Elusive spermatozoa visualized by current non-fluorescent stains may be present in difficult evidences[1]. Ten samples of vaginal, perinea and

underwear evidences with intense epithelial cell desquamation where selected from six cases out of a broader selection of sexual-assault cases. Kernechtrot-Picroindigocarmine (KPIC) stained samples with no spermatozoa previously found were confirmed sperm-negative when secondly stained by the SPERM HY-LITER™ Express method[2,3], in comparison with one KPIC sperm-positive and one semen control. Suspicious KPIC-refringency seen in some non-spermatic cells was not parallel with the specific fluorescence intensity, as they were in a lower degree of staining emission (CF488) when comparing spermatozoa nuclei as controls. In our work, SPERM HY-LITER™ stained other non-spermatic nuclei as yeast cells, present in vaginal samples, but in a lesser degree of emission intensity. Thus, intensities above background should be measured to ascertain range values for sperm nuclei identification[4]. Moreover, the difference in CF488 intensity between the sperm nuclei and the other released cells should be about 500 fold (Reich K, Independent Forensics, 2015, pers comm.), however, nuclei shapes must also be considered for selection. Image packages with fluorescent activated cell sorters would allow both intensity and shape ranges determination to accurately identify sperm nuclei.

1. Sakaida K, Williston K, Strong K and Roy R, *Searching for the elusive spermatozoa: Revisiting seminal fluid.* In: American Academy of Forensic Sciences. The Forensic Sciences, Washington DC; Feb. 18–23, 2013.
2. Miller KWP, Old J, Fischer BR, Schweers B, Stipinaite S and Reich K: *Developmental validation of the SPERM HY-LITER™ kit for the identification of human spermatozoa in forensic samples.* Journal of Forensic Sciences; 2011: 1–13.
3. Old J, Martesteck C, Kalinina A. *Developmental Validation of SPERM HY-LITER™ Express*. In: http://www.ifi-test.com/sperm-hy-litertm: Independent Forensics; 2012: 1–11.
4. De Moors A and Frégeau CJ: *Automated scoring of Sperm Hy-Liter™-stained spermatozoa by the MetaSystems Metafer image analysis software system in sexual assault specimens.* Forensic Science International: Genetics Supp. Ser. 3; 2011:e35–e36.

264. VALIDATION OF TISSUE-SPECIFIC DNA METHYLATION MARKERS FOR THE DIFFERENTIATION BETWEEN MENSTRUAL AND PERIPHERAL BLOOD

Gausterer C.[1], Mitscha-Maerheim M.[1], Stein C.[1], Egger G.[2]

[1] Forensisches DNA Zentrallabor GmbH, Medical University of Vienna, Austria
[2] Clinical Institute of Pathology, Medical University of Vienna, Austria

christian.gausterer@meduniwien.ac.at

The aim of this work was to establish PCR-based assays for the detection of DNA methylation patterns that can be exploited for the discrimination between samples originating from menstrual blood and peripheral blood, respectively. For this purpose, we evaluated selected candidate sequence motifs that are known to be differentially methylated in a tissue-specific manner. Samples of menstrual and peripheral blood from five female adult donors were harvested daily for the duration of one menses (three to five days). DNA was extracted from collected blood samples. Aliquots of DNA extracts were pooled, subjected to sodium bisulfite-conversion and analyzed via quantitative methylation-specific PCR (qMSP) following the MethyLight qPCR protocol. Pooled DNA samples (without bisulfite-treatment) were further analyzed by methylation-sensitive restriction enzyme (MSRE) PCR. Results obtained from these various analytical approaches consistently revealed quantitative and qualitative differences in the methylation status of targeted sequences between menstrual and peripheral blood samples. Based on these observations, a novel MSRE-multiplex PCR was established by combining the discriminatory singleplex PCRs with two control assays (for loading and enzymatic restriction). All test samples were investigated separately by MSRE-multiplex PCR. Obtained results were subjected to statistical analyses. Finally, the MSRE-multiplex PCR was further supplemented with primers from a commercial STR profiling kit (PowerPlex® S5, Promega). Thereby, a combined multiplex PCR (CMP) was created which allowed genotyping by STR profiling and the differentiation between samples

originating from menstrual and peripheral blood – all simultaneously in a single tube reaction.

265. IMPACT OF USING VALIDATED OR STANDARD REFERENCE GENES FOR MIRNA QPCR DATA NORMALIZATION IN CELL TYPE IDENTIFICATION

Sirker M.[1], Liang W.[2], Zhang L.[2], Rothschild M. A.[1], Gomes I.[1], Schneider P. M.[1] and the EUROFORGEN-NoE Consortium

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany
[2] Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, PR China

Miriam.Sirker@uk-koeln.de

miRNA profiling for the identification of body fluids or tissues has stimulated the interest in the field of forensic genetics. Due to their small size and low susceptibility to degradation, these molecules show advantages for casework investigations, especially regarding unknown or aged stain material. Therefore, the analysis of cell type-specific miRNA expression patterns using qPCR is the method of choice owing to its sensitivity and specificity. Despite the advantages of this technique, a proper normalization strategy is a prerequisite to obtain reliable results which is often underestimated or not considered.

With this study we aimed to demonstrate that for accurate normalization of qPCR data it is important to validate a suitable set of reference genes for specific experimental requirements instead of utilizing a common standard. For that purpose, we tested two validation strategies by comparing normalized gene expressions of tissue-specific miRNA targets against: 1) previously validated endogenous controls miR92 and miR374; and 2) U6B, a frequently used miRNA reference. The target genes chosen were miR451 and miR16 for blood, miR205 and miR658 for saliva, miR10b and miR135 for semen and miR203 for skin. All samples were tested with a TaqMan based detection system and qPCR data were analyzed using the qbaseplus software (Biogazelle). Results showed that using the validated references miR92 and miR374, the expression levels of target genes were considerably higher than using the conventional reference U6B. Furthermore, in some cases specificity of target genes was increased when applying U6B for normalization masking unspecific miRNA co-expression in other tissues.

266. REFERENCE GENES STUDY: A REQUISITE IN QRT-PCR EXPERIMENTS

Afolabi O. A.[1], Iyengar A.[1], Roeder A.[2], Hadi S.[1]

[1] School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, United Kingdom
[2] Cellmark Forensic Services, Abingdon Oxfordshire, United Kingdom

Aoafolabi@uclan.ac.uk

Reference genes are used in Forensic body fluid identification studies, involving qRT-PCR technique. These are used in order to normalise, sample-to-sample, experimental and environmental variations that might occur. In this study, 10 most common reference genes that have been published in forensic body fluid identification studies were selected from relevant literature. These included, UCE, TEF, GAPDH, 18S rRNA, ACTB, B2M, B-Actin, OAZ1, RPS 29 and S15. qPCR efficiency was tested using SYBR Green detection chemistry. Serial dilution of five commonly encountered body fluids; blood, saliva, semen, vaginal secretion and menstrual blood was prepared and Ct values were generated from the amplification curve. Five of the reference genes (UCE, TEF, ACTB, B2M, and RPS29) attained the required 95%-110% efficiency, with a standard curve slope of -3.33±10. These were further selected for Taqman assay design and then analysed on the five body fluid using the Taqman probes designed for each marker. All the markers except TEF displayed high sensitivity and were detected up to 25 picogram of RNA input. Body fluid samples were prepared on sterile cotton swabs and stored at room temperature. A time course study using the body fluid samples, showed that ACTB, RPS29 and UCE were stably expressed for 6 months across all body fluid samples. Taqman probe assays evaluated reference genes for forensic body fluid indetification, robustly demonstrating

ACTB, RPS29 and UCE as ideal markers in qRT-PCR normalisation studies. The study reieterates that reference genes should be selected upon adequate studies for their suitability for an assay.

267. DEVELOPMENT OF A RNA PROFILING ASSAY FOR BIOLOGICAL TISSUE AND BODY FLUID IDENTIFICATION

Chong K. W. Y.[1], Thong Z.[1], Wong Y.[1], Kiat Ng B.[1], Syn C.[1,2]

[1] DNA Profiling Laboratory, Applied Science Group, Health Science Authority, 11 Outram Road, 169078, Singapore
[2] Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore

chong_wai_yin@hsa.gov.sg

With the emergence of RNA profiling in the early 2000s, identifying the type of biological tissue or body fluid on forensic samples is no longer limited to conventional biochemical and serological methods. The biochemical method is presumptive in nature, while the serological method can only confirm the presence of semen, blood, and saliva on a sample. Neither method allows for 'multiplexing', i.e. concurrent detection of several different biological fluids in a single assay. RNA profiling on the other hand, is capable of distinguishing between a wider array of forensically relevant biological tissues and body fluids, such as intra-venous and menstrual blood. As such, our aim is to develop RNA profiling capability in the laboratory for biological tissue and body fluid identification.

RNA profiling couples reverse transcription polymerase chain reaction (RT-PCR) with the capillary electrophoresis of amplicons, thus permitting the multiplexing of many RNA biomarkers in a single PCR reaction. As these biomarkers are rarely specific to only one tissue/fluid type, the ability to use multiple biomarkers for the same tissue/fluid type in RNA profiling will provide greater confidence in typing a tissue/fluid. As such, multiple messenger RNAs (mRNA) and micro-RNAs (miRNA) reported in literature were assessed for their suitability as RNA biomarkers for various biological tissues and body fluids, such as blood, saliva, skin, menstrual blood, and semen. Selected RNA biomarkers were subsequently incorporated into a multiplex PCR reaction with capillary electrophoresis (CE) for RNA profiling, which will be presented here.

268. RELIABILITY OF RT-PCR FROM RNA DEGRADED SAMPLES: AN IN VITRO MODEL

Palladino R.[1], Sorçaburo Cilieri S.[1], Bonin S.[1], Pitacco P.[1], Nicolin V.[1], Previderè C.[2], Marrubini G.[3] Fattorini P.[3]

[1] Department of Medicine, Surgery and Health, University of Trieste, Italy
[2] Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy
[3] Department of Drug Sciences, University of Pavia, Italy

fattorin@univ.trieste.it

The analysis of mRNA for the identification of the source of body fluids is a challenging task in Forensic Genetics. Since the role of RNA degradation on the outcome of RT-PCR analysis is scarcely investigated, an *in vitro* system was developed here. RNA from the cell line MDA-MB-453 was hydrolyzed in water at 70 °C for 0-11.5 hours. After cDNA synthesis, duplex-qPCR analysis of two housekeeping targets (GAPDH and the PDGB) was performed. cDNA levels were then assessed both by the small amplicon format (SAF) of 60 bp and the large amplicon format (LAF) of 260 bp.

Calibration data showed linearity in the range of 1.6-420 ng of RNA for SAF and 6.6-420 ng for LAF. The Ct_{GAPDH}/Ct_{PDGB} ratio was 0.745±0.023 for SAF and 0.760±0.016 for LAF, with a trend to their increment (r^2=0.927 and, respectively, r^2=0.959) inversely related to the amount of template.

The analysis of 420 ng of hydrolyzed RNA showed that both targets were always detected, in both amplicon formats, with Ct_{GAPDH}/Ct_{PDGB} ratios of 0.719±0.008 in the SAF and 0.755±0.026 in the LAF. However, a strong increment of the Ct, related to the extent of the hydrolysis (r^2=0.904-0.988), was found.

The data of this study show that degraded RNAs represent useful templates for the accurate identification of differently expressed targets. However, since a clear trend between the increment of the CtGAPDH/CtPDGB and the length of the damaging treatment was also observed (r^2=0.790 for LAF and r^2=0.721 for LAF, respectively), the drop out of the less represented target PDGB is expected from highly degraded RNA.

269. SEARCHING FOR NEW METHYLATION SITES FOR TISSUE IDENTIFICATION

Köcher S., Teschner J., Nagy M.

Department of Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité- Universitätsmedizin, Berlin, Germany

s.koecher@gmx.de

In several crime cases it is important to determine the source of a biological tissue of a trace. In routinely work different identification tests are applicable, for example the often used immunologically protein based presumptive tests as Seratec® or RSID™. Former experiences showed some unfavourable effects in specificity, sensitivity and reproducibility of these tests. In a preliminary study we tested a new, alternative method to detect semen in biological samples with a DNA-based assay, in which tissue specific methylation patterns (TSMP) in the promoter region of suitable genes were analyzed [1, 2]. This method advantages that both analysis STR-analysis and the tissue identification are facilitated with one DNA extract. Now we are working on the identification and evaluation of additional markers for tissue-specific detection, especially to discriminate saliva. Therefore, we initially searched for proteins, which are preferably specific for a particular body fluid and designed specific primers for PCR amplification. By the application of a methylation-sensitive restriction enzyme (HAP II, restriction site CCGG) previous to PCR amplification DNA is subjected to digest, if no methylation is present at the specific locus. In those cases PCR amplification fails. For a first evaluation, PCR-products were tested by simple agarose gel electrophoresis. Most promising loci were detected afterwards with the use of labeled fluorescent-primers by capillary electrophoresis. Here we present our study of new potential markers to identify the tissue type of a DNA-sample.

1. Frumkin, D., et al., *DNA methylation-based forensic tissue identification.* Forensic Sci Int Genet, 2011. 5(5): p. 517-24.
2. Wasserstrom, A., et al., *Demonstration of DSI-semen--A novel DNA methylation-based forensic semen identification assay.* Forensic Sci Int Genet, 2013. 7(1): p. 136-42.

271. IDENTIFICATION OF BODY FLUID USING TISSUE-SPECIFIC DNA METHYLATION MARKERS

Fu X., Wu J., Wang J., Huang Y., Hou Y., Yan J.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

prof.hou@yahoo.com, forensic@scu.edu.cn

Determination of type and origin of body fluids found at crime scenes can provide important clues for forensic investigation. Recently, DNA methylation markers have proved to be a new promising tool for forensic body fluid identification. The goal of this study is to identify CpG sites that display body fluid-specific differentially methylation patterns as DNA methylation markers for forensic purpose. Using pyrosequencing technique, we examined five tissue-specific differentially methylated regions (tDMRs) for the genes BIK, CYTH4, GAS2L1, MDFI, and OSM on five different body fluids (venous blood, saliva, semen, menstrual blood and vaginal). Of the tested tDMRs, four showed different DNA methylation profiles in five body fluids ($P<0.05$). The differences were unrelated to age and gender of body fluid's carrier ($P>0.05$). A body fluid identification assay based on four body fluid-specific DNA methylation markers was set up and evaluated in 40 body fluid samples. The assay could discriminate five body fluids with high sensitivity and specificity. Our results indicated that the set of body fluid-specific CpG sites could become useful DNA

markers for forensic analysis of body fluids.

272. WHAT MICROBIAL SPECIES ARE SUITABLE FOR BODY FLUID IDENTIFICATION? AN IN SILICO ASSESSMENT USING SHORT READ DATA

Matias Rodrigues J. F.[2], Tackmann J.[2], Dobay A.[3], Haas C.[1], Kratzer A., Arora N.[1]

[1] *Zurich Institute of Forensic Medicine, University of Zurich, Switzerland*
[2] *Institute of Molecular Life Sciences, University of Zurich, Switzerland*
[3] *Institute of Evolutionary Biology and Environmental Sciences, University of Zurich, Switzerland*

Natasha.arora@uzh.ch

Accurate body fluid identification remains a challenge in forensic cases given the limitations of current identification methods such as enzymatic and immunological tests as well as mRNA profiling. Recent advances in sequencing of microbial DNA have shown that human body sites differ in their microbial communities. Since microbial cells and DNA may still be present even when human DNA and RNA are no longer found, the analyses of microbial communities open a new avenue for the identification of body fluids. In this study we capitalize on the extensive sequencing data available from previous microbial studies of human body sites to investigate the different bacterial communities present in forensically relevant body habitats including saliva, skin, vaginal fluid, semen, urine, and blood. We train and benchmark several machine learning algorithms to discriminate the microbial communities found in these body habitats, as well as to distinguish those found in human body sites from the environmental microbial communities. In addition, we identify the bacterial taxa that are most significant for the proper identification of a sample. To establish the reliability and robustness of our methods we also test these on a large and diverse set of non-human environmental microbial communities. While further experimental work on trace samples is critical to demonstrate the usefulness of microbial DNA analyses under different conditions, our results show that their application holds great potential in forensics.

273. DEVELOPMENT OF RAPID AND ON-SITE CONFORMATION SALIVA TEST BY MULTIPLEX REALTIME PCR OF THREE ORAL MICROORGANISMS

An S.[1], Park H.-C.[1], Lee K.[1], Ahn E.-R.[1], Lee Y.-H.[1], Park K.[1], Yoon H.[2], Lim S.[1]

[1] *Forensic DNA Division, National Forensic Service, 10 Ipchun-ro, Wonju-si, Gangwon-do, 220-170, South Korea*
[2] *JS Biotech, Business Incubation Center, Kyungbok University, 425 Kyungbokdae-ro, Jinjeop-eup, Namyangju-si, Gyeonggi-do, 472-948, South Korea*

pkw7777@korea.kr

Identification of saliva stain is important forensic DNA typing and bloodstain pattern analysis. Traditional saliva test methods such as SALigAE, Phadebas, and Starch-iodine gel diffusion had been used in forensic laboratories. Recently lots of studies were carried out using mRNA and oral bacteria as a confirmatory test for saliva stain. In this study, realtime quantitative PCR system was developed for analysis of saliva stain by identifying three oral bacterial strains, Streptococcus salivarius, Streptococcus sanguinis, and Neisseria subflava which were isolated from normal adults and characterized using MSID V2.1 Library Validation Statement MicroSEQ® Microbial Identification System(Applied Biosystems). PCR primers and multiplex PCR conditions were designed for amplification of species specific genes or region. Validation study for forensic case works and feasibility for expectorated bloodstain identification were also carried out. Direct protocol using portable realitme PCR machine(NBS lapChip G2-3, NanoBiosys) was designed for on-site analysis. This study was expected to contribute accurate and rapid determination of saliva on forensic biological evidence.

* Author correspondence: e-mail; neobios@korea.kr,

Phone; +82-33-902-5721, Fax; 82-33-902-5941

274. SIMPLE METHOD FOR THE IDENTIFICATION OF SALIVA AND VAGINAL FLUID BY DETECTING STREPTOCOCCUS SPECIES AND LACTOBACILLUS SPECIES

Nam Y. H.[1], Oh M.-H.[2], Moon S.-O.[1], Kim H.-S.[1], Sun S.-H.[1], Hong S.-B.[1], Kang P. W. , Han M.-S.[1]

[1] Forensic DNA Division, National Forensic Service, Won-ju 220-170, Republic of Korea
[2] Department of Nanobiomedical Science, Dankook University, Cheonan 330-714, Republic of Korea

yhnam77@korea.kr

In the sexual crimes, the identification of the sample origin is crucial for proving criminal acts, not only for the suspect's sample but also for the victim's sample. Various studies have been applied including methylation pattern analysis and the identification of the microorganisms for determination of body fluid origin. For this purpose, a novel multiplex PCR method for detection specific bacteria has been developed in this study. We target the *Streptococcus* species (*Streptococcus mutants* and *Streptococcus salivarius*) in saliva and *Lactobacillus* species (*Lactobacillus gasseri* and *Lactobacillus crispatus*) in vaginal fluid, which can provide evidence in sexual crime. Consequently, we suggest that the present multiplex PCR method is invaluable for detection of *Streptococcus* species and *Lactobacillus* species in sexual crime.

1. Kayser, M., de Knijff, P.: *Improving human forensics through advances in genetics, genomics and molecular biology.* Nat. Rev. Genet. 2011; 12: 179-192.
2. Virkler, K., Lednev, I. K.: *Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene.* Forensic Sci. Int. 2009; 188: 1-17.

275. IN-HOUSE VALIDATION OF AN RNA/ DNA CO-EXTRACTION STRATEGY FOR BODY FLUID IDENTIFICATION / STR PROFILING AND APPLICATION TO FORENSIC CASEWORK

Jucker R., Sulzer A., Voegeli P., Haas C., Kratzer A.

Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

rinaldo.jucker@irm.uzh.ch

The analysis of cell-specific mRNA expression is a confirmative method for the identification of body fluids. The European DNA Profiling Group (EDNAP) came up with a set of reliable RNA markers for the identification of the most common forensic body fluids, namely blood, saliva, semen, menstrual blood and vaginal secretion [1-3]. We used these markers for an in-house validation of an RNA/ DNA co-extraction strategy allowing for positive identification of the body fluid source of origin by mRNA profiling as well as a simultaneous identification of the body fluid donor by STR profiling. Twenty samples per body fluid were extracted and analyzed with a screening multiplex containing 2 EDNAP markers per body fluid and additionally with the body fluid specific EDNAP multiplexes. We looked at specificity, repeatability, robustness and intra/inter individual variability. DNA-quantification and STR-profiling were performed according to standard operation procedures. We applied this method to challenging forensic casework samples and report about positive and negative experiences.

1. Haas, C., et al., *RNA/DNA co-analysis from blood stains--results of a second collaborative EDNAP exercise.* Forensic Sci Int Genet, 2012. 6(1): p. 70-80.
2. Haas, C., et al., *RNA/DNA co-analysis from human saliva and semen stains--results of a third collaborative EDNAP exercise.* Forensic Sci Int Genet, 2013. 7(2): p. 230-9.
3. Haas, C., et al., *RNA/DNA co-analysis from human menstrual blood and vaginal secretion stains: Results of a fourth and fifth collaborative EDNAP exercise.* Forensic Sci Int Genet, 2014. 8(1): p. 203-12.

Theme 11: Touch DNA

276. DNA PROFILES FROM FINGERMARKS

Templeton J. E. L.[1], Blackie R.[1], Taylor D.[1,2], Handt O.[1,2], Linacre A.[1]

[1] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[2] *Forensic Science South Australia, Adelaide, Australia*

adrian.linacre@flinders.edu.au

The ability to generate a DNA profile from a fingerprint for the purpose of human identification will have significant implications for solving a broad spectrum of criminal investigations, ranging from theft to crimes of violence. DNA retrieved from fingermarks deposited by touch (referred to as 'touch' DNA) is often degraded, limited in quantity and may comprise elements that co-extract with the DNA and hinder subsequent amplification. Standard protocols for swabbing and extracting 'touch' DNA often recover sub-optimal levels of trace nuclear DNA that can result in a poor quality STR DNA profile or no profile at all. In particular, the ability to recover identifiable DNA from certain substrates, such as brass cartridge cases, has proven to be extremely difficult using current methodology. Here, we report a novel application that can routinely generate informative DNA profiles from latent fingerprints using standard STR DNA profiling kits, without the need for enhanced PCR cycle number[1]. Its novelty that will be discussed involves an optimised swabbing technique and detergent medium, omission of a DNA extraction process and the addition of PCR facilitators to the reaction vessel. By using a direct PCR approach we can routinely generate full DNA profiles from fingerprints that have been deposited 15 minutes after a person has washed their hands. There is no increase in cycle number and the methodology described can easily be adapted into mainstream forensic practice. Novel and valuable applications of direct PCR to forensic practice will be discussed.

1. J.E.L Templeton & A. Linacre, DNA profiles from fingermarks, Biotechniques (2014) 5: 259-266.

277. CONTAMINATION IN OBTAINING TRACE EVIDENCE – AN ISSUE MORE TOPICAL THAN EVER?

Neuhuber F.[1], Pickrahn I.[1], Dunkelmann B.[1], Müller E.[1], Kreindl G.[1], Zahrer W.[1], Cemper-Kiesslich J.[1]

[1] *Institute of Legal Medicine, University of Salzburg, Austria*

franz.neuhuber@sbg.ac.at

Contamination of biological trace material by police officers is a never-ending issue in forensic trace analysis. For Austria a study from the year 2010 (1) reported a confirmed contamination rate of 0.36% (90 contaminations in approx. 25,000 traces) in the federal states of Salzburg and Upper Austria. Even at that time the actual figure was estimated to be approx. 0.5%. By establishing a national "Police Elimination Database" (PED) in 2009 the number of detected contaminations in the test period 2000 – 2009 was increased from 0.36% to 0.51% (127 contaminations in approx. 25,000 traces). For the period 2010 – 2014 a current figure of 0.87% has now been determined (131 contaminations in approx. 15,000 traces).

In the Austrian National DNA Database the PED has so far helped to detect 542 contaminations in a dataset of approx. 57,000 tested traces, which amounts to a contamination rate of approx. 1%. Of 4,191 police officers recorded in the PED, 349 caused contamination at least once. Mixed traces only account for a small percentage because they are only compared if there is good reason to do so or if there is a suspicion.

Since 2013 the DNA Laboratory of Legal Medicine Salzburg has been using an additional tool to detect potential contamination, the "Profile Comparison" function of the GeneMapper™ software (Life Technologies), which has proved to be advantageous especially in the case of complex mixtures. As a result it was possible to detect numerous contaminations that hitherto would have remained undiscovered if the PED had only been used on its own. The apparently rising contamination rate over the years is possibly explained not so much by an actual increase in the number of contaminations but by improved

measures taken to detect them. At present the contamination rate of biological traces nationwide is estimated to be approx. 1% -2%.

1. Neuhuber F, Cemper-Kiesslich J, Dunkelmann B, Grabner W, Höckner G, Klausriegler E, Radacher M, Tutsch-Bauer E: Erfahrungen mit Kontaminationen in der Analyse biologischer Spuren. Kriminalistik 6 (2010), 382-384.

278. EFFECT OF TWO DIFFERENT SWABS ON GENETIC PROFILING OF ENHACED FINGERPRINTS

Tozzo P., Giuliodori A., Ponzano E., Caenazzo L.

Laboratory of Forensic genetics, DMM, University of Padova – Padua - Italy

pamela.tozzo@gmail.com

Latent fingerprints are not commonly used for DNA typing even if they could be considered useful DNA sources, as reported in literature in previous publications.

Different studies have proved that skin contact can transfer enough DNA for successful STR typing, the success rate depends on the individual handler, which hand has been used, the activities of the individual prior to touching the object and the handled substrate. In the present study we investigate the effect of fingerprint-enhancement powder methods on subsequent STR profiling from bloodstains and latent fingerprints. The research was conducted into two phases. First we performed a study typing blood traces deposited on five different surfaces, treated with eight types of dactyloscopic powders and recovered with two different type of swabs, using three different DNA extraction methods. In the second part of our study we analyzed latent fingerprints on the same five surfaces enhanced with the eight different powders used in the first part of the study. The results obtained show that the powders used in latent fingerprints enhancement, rather than having a direct inhibitory effect on extraction and amplification of DNA, may cause partial degradation of DNA, thereby reducing the efficiency of amplification reaction. The higher number of complete profiles obtained with glass and metal surfaces, suggested that their characteristics facilitate the recovery of DNA. Despite these results, it should not be forgotten that they were obtained under laboratory conditions and in real caseworks there may be still different problems involved.

279. DNA EXTRACTION FROM FORENSIC TAPE LIFTS - A COMPARISON OF TWO DIFFERENT METHODS

Joël J., Glanzmann B., Germann U., Cossu C.

Institute of Forensic Medicine, Kantonsspital St. Gallen, St.Gallen, Switzerland

Jana.Joel@kssg.ch

In addition to swabbing and cutting, taping became an important method to collect DNA from crime scene items. Many studies with different kind of tapes, substrates and traces were previously published. However, the influence of the extraction method on the DNA recovery has been less focussed on. The aim of this study was to test the DNA recovery from Scensafe Fast Tapes extracted with the PrepFiler Express BTA (Applied Biosystems) and Chelex/iPrep (Invitrogen). Preliminary tests showed difficulties during lysis since the PrepFiler Express BTA with a maximum of 230 µl lysis buffer did not cover an intact tape. Therefore, halves of the tapes were subsequently used. The highest DNA amounts were extracted when half-tapes were not cut into pieces in order to avoid sticking together and contamination. The study showed higher DNA yields for every step of a serial dilution of human blood when extracted with PrepFiler Express BTA compared to the extraction with Chelex and iPrep: BTA-extracted samples of dilutions 1:10, 1:50 and 1:100 reached 2.2, 1.8, 3.1 times higher DNA amounts respectively; BTA-extracted samples of dilutions 1:500 and 1:1000 showed 9.6 and 15 times more DNA than the Chelex/iPrep-extracted samples. There was no DNA detected for dilutions 1:5000 and 1:10000 with Chelex/iPrep, whereas BTA-extraction obtained enough DNA for full NGM Select profiles. A statistical analysis comparing the variation of BTA extraction of 2 dilutions (1:100, 1:1000; n=10 each) revealed comparable

RSDs for both dilutions. Results of mock traces (touch DNA) are pending at the time of abstract submission.

280. DNA RECOVERY FROM SLOUGHED EPITHELIAL CELLS: A RETROSPECTIVE ANALYSIS

Colussi A., Bozzo W., Laborde L., Ortiz M., Pilili J., Carini G., Lojo M. M.

Laboratorio de Análisis Comparativo de ADN, Asesoría Pericial Departamental La Plata, Dirección General de Asesorías Periciales, Suprema Corte de Justicia de la Provincia de Buenos Aires, Argentina

agcolussi@gmail.com

The improvement in the sensitivity of DNA profiling techniques has expanded the number of evidence type, including samples taken from touched surfaces. This feature allowed the incorporation of misdemeanors (such as robbery and theft), increasing the workload. Currently, almost 18.5% of requested analysis entered in our laboratory includes these kinds of samples, which required an isolated laboratory environment and optimized experimental procedures in order to avoid contamination with foreign DNA. Under such conditions DA was extracted either with QIAamp DNA Mini (QIAGEN), DNA IQ System (Promega) and AutoMate Express DNA Extraction System (Applied BioSystems) BTA protocols of extraction.

The retrospective analysis of these kinds of evidences showed a gradient with regard to the DNA yield and the quality of the obtained profile that could be associated with the transfer mechanism of the epithelial cells. The analysis of the results obtained from 1050 different evidences taken as a whole showed that informative DNA profiles were obtained in only 23% of the analyzed samples; ranging from ropes, seals and laces to clothes.

282. PERSISTENCE AND SECONDARY TRANSFER OF DNA FROM PREVIOUS USERS OF EQUIPMENT

Fonneløp A. E.[a,c], Johannessen H.[a], Gill P.[a,c]

[a] *Norwegian Institute of Public Health, Oslo, Norway*
[c] *University of Oslo, Oslo, Norway*

ane.elida.fonnelop@fhi.no

Explanations to describe how a defendant's DNA came to the crime scene are often questioned in court. Previous studies have demonstrated that DNA transfer to an object, such as a computer keyboard, can persist for some time after it has been passed on to a new user. In this study, we investigated the possibility of DNA transfer from the initial user of equipment to a new user in a daily setting (continually used). We also investigated persistence - how long after receiving the object was it possible to transfer DNA to the new user's hands? Four individuals (two male and two female) swapped their personal computer keyboard and mouse. One of the participants was previously known to be a good shedder. Samples were collected from parts of the equipment (background DNA), and from the new users hands on the first day and then frequently until the previous owners' DNA could not be detected. Additional Y-analysis was performed on the samples collected from the female participants' hands. In the samples collected on the first day of the swap, alleles identical to those of the initial user were observed in mixtures in all cases except the samples collected from the good shedder hands. In the samples collected 7 days after the swap, alleles from the initial user could still be detected as a small contributor to the mixture. In the samples collected three weeks after the swap, it was not possible to identify the initial user from standard analysis.

283. REFERENCE MATERIAL FOR COMPARISON OF DIFFERENT ADHESIVE TAPES FOR FORENSIC DNA SAMPLING

Forsberg C.[1], Wallmark N.[1], Hedell R.[1,2], Jansson L.[3], Ansell R.[1], Hedman J.[1,3]

[1] *Swedish National Forensic Centre, Linköping, Sweden*
[2] *Department of Mathematical Sciences, Chalmers University of Technology and University of Gothenburg, Gothenburg, Sweden*

[3] *Applied Microbiology, Lund University, Lund, Sweden*

christina-u.forsberg@polisen.se

Tape-lifting is an efficient method for collecting traces of cellular material from fabrics. Since 2006, an in-house adhesive tape has been used in casework at the Swedish National Forensic Centre, Linköping. Although this tape gives good DNA yields, we aim to replace it with a commercial tape to save cost and labor. In order to enable a fair comparison between different adhesive tapes, we have developed and evaluated a method for production of relevant reference material. One person, known to be a good shedder, wore identical long-sleeved T-shirts under controlled circumstances, and trace recovery was systematically performed with the in-house tape (3 T-shirts, total of 24 samples). Each sample was DNA extracted (Chelex) and quantified (Quantifiler Human DNA Quantification kit) to find the normal variation within the reference material. The DNA recovery differed considerably between samples, with obtained DNA concentrations between 0.010-0.481 ng/µL (mean: 0.083, standard deviation: 0.116 ng/µL). Applying such a reference material for comparison between two commercial tapes and our in-house tape resulted in mean DNA recoveries plus/minus one standard deviation of 0.013±0.006 ng/µL (Scenesafe FAST Box), 0.012±0.007 ng/µL (Touch Tape), and 0.023±0.013 ng/µL (in-house tape). The in-house tape gave statistically significant higher yield compared to Touch Tape ($p<0.05$), but for Scenesafe the difference was not significant. Shedding of cells to worn clothes is a random process. Having a systematically prepared, casework-like reference material with known variation is therefore vital for comparative studies of tapes.

284. VECTORS OF DNA TRANSFER IN A LABORATORY ENVIRONMENT

Taylor D.[1,2], Abarno D.[1,2], Rowe E.[2], Rask-Nielsen L.[3]

[1] *Forensic Science South Australia, 21 Divett Place, Adelaide, Australia*
[2] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[3] *School of Biological Sciences, Adelaide University, Adelaide, Australia*

Duncan.Taylor@sa.gov.au

Mechanisms of DNA transfer and deposition are of significant interest to investigators and the court system. Often in court a scenario will be posed to the scientist asking the possibility (or probability) that it could explain the results obtained. A scientist's ability to provide assistance to the court is limited by the sparsity of information relating to the various proposed senarios and information about factors such as transfer rates, transfer types and DNA persistence. Transfer studies largely require a set of controlled conditions in order to better elucidate the relationship between cause and effect and consequently there is a limit to the extent to which these results can be applied to DNA transfer in uncontrolled environments. In this study objects and furishings located within one floor of a building, comprised office and laboratory space, were sampled to examine the presence of DNA in both public and restricted areas. We compared the DNA profiles obtained to reference profiles from consenting staff members who frequent areas of the floor. Analysing the results allowed us to track the permeation of individuals' DNA throught the building in a semi-uncontrolled environment. This allows us to draw some general conclusions regarding topics such as secondary transfer, shedder status and persistence of DNA and inform interpretation of DNA transfer in uncontrolled environments.

285. SELF VERSUS NON-SELF IN HANDPRINTS

Goray M.[1], Szkuta B.[2,3], van Oorschot R. A. H.[3]

[1] *Victoria Police Forensic Services Department, Melbourne, Australia*
[2] *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia*
[3] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia*

bszk@deakin.edu.au

When an individual's hands come into contact with a previously used item or surface, in most instances they will deposit their own DNA while collecting foreign or 'non-self' DNA which may have been present on that item/surface

in the bidirectional transfer. Whilst the deposition of 'self' DNA has been shown to vary depending on the portion of the hand making contact, the manner of handling, the "shedder status" of the individual and the substrate contacted, the frequency at which non-self DNA is picked-up and deposited is rarely considered. It is believed that this non-self DNA is present on everyday surfaces and objects from previous contacts by other individuals, yet there has been little consideration of the relative portions of 'self' and 'non-self' DNA deposited in a single touch. In order to evaluate the proportion of self versus non-self DNA deposited in a single contact, over 200 full handprints left on glass, on a number of independent occasions, were assessed for the type of DNA result obtained (self or non-self) as well as mixture proportions of self to non-self DNA, where applicable. Mixed profiles were observed in more than 50% of the prints. While in many instances the depositors (self DNA) was the major contribution to the mixture, there were instances where the non-self DNA proportion was greater than that of the depositor. During routine daily activity DNA is readily picked-up and transferred from and to objects and the depositor is the major contributor to the DNA detected in most instances.

286. RISK OF DNA TRANSFER BY GLOVES IN FORENSIC CASEWORK

Margiotta G.[1], Tasselli G.[2], Tommolini F.[2], Lancia M.[2], Massetti S.[2], Carnevali E.[2].

[1] Section of Legal Medicine, University of Siena, Italy
[2] Section of Legal Medicine, University of Perugia, Italy

gabrielemargiott@hotmail.com

INTRODUCTION: In the last years, the increased sensitivity of a new generation of STR kits have contributed to a stronger focus on the issue of contamination (1) . DNA contamination can occur by either direct or indirect transfer. Direct transfer includes contact, but also activities within the vicinity of an item that may result in the transfer of DNA from an individual without any contact, such as speaking or coughing. Indirect transfer of DNA is when DNA from an individual comes to be on an item via an intermediary surface (2). Contamination within the laboratory may occur as a result of unprotected speaking, through the presence of DNA on unused laboratory gloves, and ineffective cleaning procedures (3). A recent study noted the transfer of dried blood from and to cotton via tools used during examination(scissors, forceps and gloves), and indicated that these instruments pose a high contamination risk if they are not adequately cleaned (4). In our work, we analyzed all the gloves used in one day by four operators working in our laboratory. For every glove, we evaluated the presence of contamination DNA from the operator or from other samples. The results are presented here. CONCLUSION: In forensic casework, it's imperative to change gloves every time after touching items or surfaces, prior to touching the exhibit. It's desiderable to wear multiple layers of gloves to avoid skin exposure during the changing of gloves.

1. Neureuther K, Rohmann E, Hilken M, Sonntag ML, Herdt S, Koennecke T, Jacobs R, Adamski M, Reisbacher S, Alfs K, Strain P, Bastisch I. Reduction of PCR-amplifiable DNA by ethylene oxide treatment of forensic consumables. Forensic Sci Int Genet. 2014 Sep;12:185-91.
2. Meakin G, Jamieson A. DNA transfer: review and implications for casework. Forensic Sci Int Genet. 2013 Jul;7(4):434-43.
3. Szkuta B, Harvey ML, Ballantyne KN, van Oorschot RA. DNA transfer by examination tools - a risk for forensic casework? Forensic Sci Int Genet. 2015 Feb 16;16C:246-254.
4. Szkuta B, Harvey M.L, Ballantyne K.N, van Oorschot R.A.H, The potential transfer of trace DNA via high risk vectors during exhibit examination, Forensic Sci. Int.: Genet. Suppl. Ser. 4 (1) (2013) e55–e56.

287. EVALUATION OF COLLECTION AND EXTRACTION METHODOLOGIES OF LATENT FINGERPRINTS FOR MILITARY APPLICATION

Oliveira T. P.[1,2], Nogueira T. L. S.[1,2], Braz E. B. V.[1], Santos O. C. L.[1], Carvalho E. F.[2], Silva D. A.[2]

[1] *Instituto de Biologia do Exército, Rio de Janeiro, Brazil*
[2] *Laboratório de Diagnósticos por DNA, DECOL, IBRAG, Universidade do Estado do Rio de Janeiro, Brazil*

dayse.a.silva@hotmail.com

Latent fingerprint have been commonly used by Brazilian Army forensic experts as an effective tool for personal identification in crime scene investigations. In cases in which samples are considered unusable for papiloscopy, genetic analysis appears as a complementary method that prevents waste of expert testimony. The aim of our study was to standardize DNA collection and extraction techniques in two sorts of surfaces: glass and metal, in order to adapt these methodologies to military reality. Brazilian Military fingerprints were collected using two swabbing solutions: NaCl 0.9% and Sodium Dodecyl Sulfate 0.01% (SDS). DNA was isolated by testing three kinds of methods: Chelex 5% resin, Lysis Solution 0.05% SDS and Proteinase K 20 mg/mL and QIAmp® DNA Investigator® Kit (Qiagen), according to the manufacturer's instruction. After the extraction, PCR amplification was performed with AmpFlSTR® Identifiler® Amplification Kit (Life Technologies). Amplified products of STR multiplexes were electrophoresed on ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems) and the data was analyzed using GeneMapper® ID Software v3.2 (Applied Biosystems). Although further tests need to be performed, the results have shown that Chelex resin is not suitable for recovering DNA from fingerprints on glass and metallic surfaces. It was observed that QIAmp® Investigator® DNA Kit (Qiagen) and Proteinase K extraction gave satisfactory results for samples collected both on glass and metallic supports. Furthermore, it has been shown that the two collection solutions employed contributed to the appearance of genetic profiles, which makes these methodologies applicable for large-scale analysis.

1. Alberink I, Jongh A, Rodriguez C. Fingermark evidence evaluation based on automated fingerprint identification system matching scores: The effect of different types of conditioning on likelihood ratios. J Forensic Sci. 2013; 59(1): 70-81.
2. Kishore R, Hardy WR, Anderson VJ, Sanchez NA, Buoncristiani MR. Optimization of DNA extraction from low-yield and degraded samples using the BioRobot® EZ1 and BioRobot® M48. J.Forensic Sci. 2006; 51(5): 1055-1061.
3. Lehmann VJ, Mitchell RJ, Ballantyne KN, Oorschot RAH. Following the transfer of DNA: How far can it go?. Forensic Sci Int. 2013; 4(2013): 53-4.

288. ACTIVITIES BETWEEN ACTIVITIES OF FOCUS – RELEVANT WHEN ASSESSING DNA TRANSFER PROBABILITIES

van Oorschot R. A. H.[1], McColl D. L.[1,2], Alderton J. E.[1,3], Harvey M. L.[2], Mitchell R. J.[3], Szkuta B.[1,2]

[1] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia*
[2] *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia*
[3] *Department of Genetics, La Trobe University, Melbourne, Australia*

roland.vanoorschot@police.vic.gov.au

Consideration of likelihoods of various indirect transfer scenarios, as opposed to direct transfer scenarios, is increasingly requested in case deliberations. Deliberations of indirect transfer scenarios often focus on the initial activities when the DNA in question is being picked up, and on the activities surrounding its deposition at the location it is sampled from. There is, however, frequently a time between these activities and insufficient attention is given to activities that may occur during this period, or the impact they may have on profiles generated.

To understand the frequency and manner of what items/surfaces are touched during a range of everyday activities, we observed human behaviour during a variety of activities and assessed factors including: what is being touched and how, the duration and frequency of contact, the personal or non-personal nature of what is being contacted and the recent history of the objects touched.

Our findings show that during various general activity scenarios a variety of items and surfaces are frequently contacted over relatively short periods of time.

Appreciation and consideration of general activities that may occur between key focus activities (i.e. those associated with the pickup of DNA in question at point A and deposit at collection site B) and their potential impact on what is deposited at the final collection site, are imperative to assisting weighting of alternative transfer scenario propositions.

289. SHEDDER STATUS – REALITY OR MYTH?

Goray M.[1], Szkuta B.[1,2], van Oorschot R. A. H.[3]

[1] *Victoria Police Forensic Services Department, Melbourne, Australia*
[2] *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia*
[3] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia*

roland.vanoorschot@police.vic.gov.au

There are several studies suggesting that people deposit different quantities of their own DNA on the items they touch, with some indicating that there are good shedders and bad shedders. This belief has been perpetuated by the knowledge that some people deposit larger or smaller amounts of DNA when they touch items/surfaces, however, it is of interest to determine whether they do so consistently.

To establish whether shedder status is reality or myth, several individuals were tested for their ability to deposit DNA by placing full handprints on a clean glass plate at three set times during the day on several days over several weeks. Some of the participating individuals were believed to be good or bad shedders prior to participation based on a range of prior relevant experiences.

Over 200 samples were collected from 10 individuals. Differences in deposit quantities were assessed along with the types of DNA profiles obtained at different times of the day and on different days, between hands of the same individual as well as between individuals. The potential influences of the participants' activities prior to depositing, their gender and hand size were also considered. Furthermore, the correlation between the deposit quantity and the ratio of self to non-self DNA in the mixed deposits were analyzed to determine if the amount of non-self DNA has an effect on overall DNA quantities. Results indicate that shedder status is a complicated issue; partly reality and partly myth.

290. RESIDUAL DNA ON EXAMINATION TOOLS FOLLOWING USE

Szkuta B.[1,2], Harvey M. L.[2], van Oorschot R. A. H.[1]

[1] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia*
[2] *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia*

bszk@deakin.edu.au

Recent studies observing the transfer of DNA via examination tools used within forensic laboratories (scissors, forceps and gloves) have highlighted the contamination risk of such implements if protocols following their use and replacement are not adhered to1. Whist these previous studies focus primarily on the transfer of biological substances to a substrate via high-risk vectors, this investigation considers the proportion of DNA that remains on the high-risk vectors following contact with the substrate. Mock forensic casework exhibits comprised of cotton or glass (gloves only) substrate deposited with dried blood or touch DNA. Following primary contact with the mock exhibit, a secondary DNA-free substrate was similarly contacted by the vector. Combinations of singular and multiple (8) contacts were applied, with each contact lasting 3 sec. Immediately following contact with the secondary substrate, the vector was

swabbed using the wet/dry method. Residual DNA was detected on the vectors in most instances, however this was dependent on the vector, substrate and biological substance applied. The results demonstrate the potential for inter- and intra-exhibit contamination through further contacts.

1. Szkuta B, Harvey ML, Ballantyne KN and van Oorschot RAH: DNA transfer by examination tools - a risk for forensic casework? Forensic Science International: Genetics. 2015; 16: page 246 – page 254.

291. PERSISTENCE OF TRACE DNA ON BURGLARY RELATED OBJECTS

Pfeifer C.[1], Frenzel J.[1], Miltner E.[1], Wiegand P.[1]

[1] *Institute of Legal Medicine, University Ulm, Ulm, Germany*

celine.pfeifer@uniklinik-ulm.de

When relevant touched items are found at a burglary crime scene STR profiles can lead to suspects. These might recognize their personal belonging but may also testify that they lost it prior to the crime and some other person has to have committed the crime. In order to acquire a better understanding of the persistence and replacement of trace DNA in those special scenarios we conducted two separate experiments:

In the first experiment typical burglary tools were owned by a first person and then used for a burglary related action by a second person, either with or without gloves. For the second experiment worn clothing and bags of participants were changed among each other and worn for 30 - 90 minutes.

Depending on the nature of contact and the surface type of tool handles we observed a more or less complete replacement of the profile of the 1st user through the 2nd user if not wearing gloves: when participants had to break a door imitating construction we never found an individual profile of the 1st user. His alleles only were discovered in mixture profiles in 24% of the cases. When the 2nd user wore gloves the 1st user was identified at least as the major contributor on the tool handle in 26% of cases (mainly low template DNA). Contrary to the tool handles clothing showed a rather complex spectrum of replacement and persistence. With this study we wish to complement the still few findings about persistence of DNA.

Theme 12: DVI Human Remains

292. DNA IDENTIFICATION OF THE VICTIMS OF MALAYSIA AIRLINES FLIGHT MH17 CRASH

Kal A., van Dongen C., van Kooten C., Slooten K., Pouwels M., Bink R., Slagter M., Kloosterman A.

Netherlands Forensic Institute, Laan van Ypenburg 6, 2497 GB The Hague, The Netherlands

a.kal@nfi.minvenj.nl

On the 17th of July 2014, Malaysia Airlines flight MH17 from Amsterdam to Kuala Lumpur crashed in the Ukraine. No one survived the crash, of the 298 victims 196 were Dutch nationals. A large number of victims were closely related to each other, which makes identification extra challenging. On 20th of July 2014 it was decided that The Netherlands would coordinate the victim identification process. Three days later the Netherlands received the first shipments of human remains from the Ukraine. DVI teams from different countries assisted in the identification process of these human remains. The DNA research in the DVI process was performed by the NFI. Our procedures, results and lessons learned will be presented at the conference.

Until the beginning of April, the NFI received the following samples for DNA-typing and matching;

- approximately 1900 samples (bone marrow swabs, soft tissue, bone, tooth) of body parts,
- approximately 350 personal items (clothes, razor blades etc.) belonging to the missing persons,
- approximately 300 reference samples (mostly buccal swabs) from relatives of the missing persons,
- approximately 200 DNA profiles of missing persons and/or relatives of missing persons typed in foreign labs.

DNA profiles of human remains were matched using the Bonaparte DVI software (www.dnadvi.nl) to the missing persons. Until the beginning of April DNA-profiles of almost all body parts where obtained which were all matched to the missing persons.

293. POLISH GENETIC DATABASE OF TOTALITARIANISM VICTIMS

Ossowski A.[1], Kuś M.[1], Zielińska G.[1], Kupiec T.[3], Stefaniak M.[2], Bykowska M.[1], Jasiński M. E.[4], Ciechanowicz A.[1]

[1] Department of Forensic Genetics, Pomeranian Medical University in Szczecin, Poland
[2] Institute of National Remembrance, Szczecin, Poland
[3] Institute of Forensic Research in Krakow, Poland
[4] Institute of Archaeology and Studies of Religion Norwegian University of Science and Technology Trondheim, Norway.

andrzej.ossowski@pum.edu.pl

In this publication we want to present the Polish Genetic Database of Totalitarianism Victims (PBGOT, www.pbgot.pl). Our aim is to use the latest methods of forensic genetics to identify the victims of totalitarian systems. The project assumes to obtain proper reference material from family members of victims of communist and Nazi crimes and due to the time passage this has become the most important task for PBGOT. Work includes also securing and storing biological material collected during the exhumation of the victims, and carrying the genetic identification process. The next task is collecting all available historical data of killed and missing people. All these procedures were already applied in practice during identifying victims of communists buried on Powązki Military cemetery in Warsaw. At this cemetery hundreds of people were buried that had been killed through military court orders during the years 1948-1956. Up to now team exhumed remains of 196 people. Another place of work was a garden next to the prison in Bialystok. At this place hundreds of people were buried after being murdered by Soviets, Nazis and the polish

Communists during the years 1939-1956. The number includes the Polish resistance movement and civilians: Polish, Russian, Jewish and Gypsies. Up to now team exhumed remains of 290 people. A team of specialists in the fields of history, archeology, forensic anthropology, forensic medicine and genetics take part in this research. The main goal is to identify the victims and so far, remains of more than 40 exhumed people were identified.

294. IMPACT OF FIXATIVES ON QUALITY AND QUANTITY OF NUCLEIC ACIDS IN ARCHIVED FORMALIN FIXED TISSUES

Josefiova J.[1], Matura R.[1], Vanek D.[1,2]

[1] Forensic DNA Service, Prague, Czech Republic
[2] Charles University in Prague, 2nd Faculty of Medicine, Prague, Czech Republic

daniel.vanek@dna.com.cz

Tissue fixation is a process of rapid protein denaturation via chemical reagents and physical modalities. The aim of fixation is to prevent spontaneous autolytic cell and tissue processes and thus preserve cell structures and body tissues as close to their natural state as possible. Fixed tissues are mainly used for histological staining, imuno-histochemical staining, in situ hybridization, mass spectroscopy and recently for DNA and RNA isolation, and their downstream analysis. Formaldehyde is the most prevalent fixative to this day. Qualities of formaldehyde, fixative's volume and time of fixation all have impact over quality and quantity of isolated nucleic acids. Different human body tissues were fixed under various conditions: 4% non-buffered or buffered formaldehyde, 4% non-buffered or buffered formaldehyde both with addition of formic acid and 36% non-diluted and non-buffered formaldehyde. The quality and quantity of extracted DNA samples was measured using SYBR-green based quantitative Real-Time PCR assay employing different amplicon sizes. The highest yield of relatively non-degraded DNA was obtained from tissues fixed in 4% buffered formaldehyde, on the other hand only small quantities of degraded DNA were obtained from tissues fixed in 4% formaldehyde with addition of formic acid.

295. A COMPARATIVE STUDY BETWEEN MUSCLE, CARTILAGE AND BONE SAMPLES FOR DNA TYPING IN DISASTER VICTIM IDENTIFICATION (DVI)

Ferreira S. T. G.[1,2], Garrido R. G.[3], Paula K. A.[1], Nogueira R. C.[4], Oliveira E. A.[4], Moraes A. V.[1]

[1] Forensic DNA Research Institute, Federal District Civil Police, Brasília, Brazil
[2] National Secretariat of Public Security, Ministry of Justice, Brasília, Brazil
[3] Institute of Research and Expertise in Forensic Genetics, Civil Police of the State of Rio de Janeiro, Rio de Janeiro, Brazil
[4] Institute of Legal Medicine, Federal District Civil Police, Brasília, Brazil

samueldoc83@yahoo.com.br

In disaster victim identification (DVI), the quality and kind of samples for DNA typing play a crucial role in the identification of the victims. The choice of samples for DNA typing depends on the conditions of the bodies and on many factors related to the incident. In this study, we compared DNA yields and DNA profiles of 20 deep red muscle samples with 20 cartilage samples from undamaged joints (knees) with 20 bone samples (phalanges from hallux) collected from the same victims of the biggest natural disaster in Brazil in which there were 918 deaths. This mass fatality incident was due to floods and mudslides in the mountainous region of the State of Rio de Janeiro in Brazil in January 2011. This study was initially performed with the analysis of cartilage samples and bone samples. Now we compare the results of the analysis of 20 red muscle samples with the results of the analysis of cartilage and bone samples. In this work, sample collection was performed with disposable equipment. We used modified organic phenol-chloroform for DNA extraction, Plexor HY Quantification Kit (Promega Corporation) to quantify the DNA in a 7500 Real-Time PCR System (Applied Biosystems) and PowerPlex Fusion Kit (Promega Corporation) and Identifiler Plus (Life Technologies) for the amplification. PCR products were analyzed on an 3130xl Genetic Analyzer (Applied Biosystems).The analysis of the results showed that cartilage and bone samples had higher amount of DNA recovered and better quality of STRs

profiles obtained than deep red muscle samples.

296. POSTMORTEM DEGRADATION OF TEETH; IMPLICATIONS FOR DNA IDENTIFICATION OF HUMAN REMAINS

Higgins D.[1], Austin J. J.[1]

[1] *Australian Centre for Ancient DNA and Environment Institute, University of Adelaide, South Australia, 5005, AUSTRALIA.*

denice.higgins@adelaide.edu.au

Of key concern for identification of skeletal remains is the limited yield of DNA from these poorly preserved tissues and the challenging form and composition of the tissues themselves. Currently the kinetics of postmortem degradation of DNA within teeth and bones is poorly understood making the formulation of ideal sampling and DNA extraction protocols problematic. We present the findings of a controlled study of the decomposition of human teeth over a 16 month period and highlight the implications of these findings for successful retrieval of DNA from these tissues. Using histology, we examine the effects of postmortem decomposition on the structure of the tooth tissues (pulp, dentine, cementum), revealing the rapid loss of pulp tissue and the breakdown of the dentine from the pulp tissue interface out. Following this, we isolate individual tissues to explore nuclear and mitochondrial DNA preservation within each. Our results demonstrate an uneven DNA distribution and inconsistent rates of decomposition across different tissues. Cementum contained the highest quality and quantity of nuclear DNA, whereas coronal dentine had low quantities of both nuclear and mitochondrial DNA. To further maximize the recovery of DNA from the mineralised tissues, we examined the use of low volume demineralisation techniques during DNA extraction, showing a clear improvement in STR profiling results from degraded cementum samples. Together, this knowledge enables a targeted sampling approach and optimized DNA extraction method to retrieve maximal DNA quantity and increase the success rate of DNA identification from skeletal remains.

297. DEVELOPMENT OF AN EVALUATION-FORM FOR AN IMPROVED DATA EXCHANGE IN THE FIELD OF DNA EXTRACTION FROM SKELETON REMAINS

Rothe J., Nagy M.

Department of Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité-Universitätsmedizin, Berlin, Germany

jessica.rothe@charite.de

One of the most demanding DNA extractions is that one from bones and teeth. Reasons for this are basically the robustness of the material and the usually low DNA content. The probably biggest challenge can be found by the manifold nature of the material, which is defined by various factors like age, storage, environmental conditions and contamination with inhibitors. However, most of the published protocols do not distinguish between different types or quality of the bone material but are described as general applicable.

In our laboratory we work with two different extraction methods based either on silica beads or the use of silica membranes. Although these two methods are based on the same chemistry, we found big differences in the DNA-yield and co-extraction of inhibitors. Related to our own experiences it has to be considered that each bone material needs its own consideration of analysis and extraction method. Hereby the most ambitious task is the valuation of the quality of the bone material, which requires substantial experiences. Therefore, we think that the exchange of data and experience is of great importance in the field of DNA extraction from bones and teeth.

In cooperation with other research-groups we developed a bone evaluation-form, which documents the state of sample preservation, the particular extraction method and the final results. Finally we aim to compose a bone-extraction catalogue, which summarizes the collected data and experiences.

298. CMP CYPRUS GENETICS UNIT: WORKING WITH CHALENGING CASES

Papaioannou K.[1], Zorba G. K.[1], Socratous E.[1]

[1] *CMP Anthropological Laboratory, United Nations Protected Area, Nicosia, Cyprus*

katerina.papaioannou@cmp-cyprus.org

The Committee on Missing Persons (CMP) in Cyprus was established in 1981 and its aim is to recover, identify and return the remains of 2001 individuals (493 Turkish Cypriots and 1,508 Greek Cypriots) who went missing during the inter-communal fighting of 1963-64 and the events of 1974 (1). The Genetics Unit of CMP was established in 2012 and consists of 1 Greek-Cypriot and 1 Turkish-Cypriot geneticist who are responsible for the interpretation and confirmation of the results received from the contracted DNA laboratory. The Genetics Unit works in close cooperation with the bi-communal team of archaeologists and anthropologists combining genetic and non-genetic data in order to reach an identification. In projects like this where the group of missing involves individuals closely related to each other the identification process is challenging. The bi-communal team of geneticists have to deal with cases in which the individuals recovered from a burial is a family of seven and the relatives available to use as reference samples are not enough to reach the identification threshold. In addition the post mortem and ante mortem information is too limited to help distinguish between the family members. On the other end, in cases where the post mortem information recovered from the remains is strong indicating the missing person's identity, the identification threshold for an inclusion cannot be reached even though there are adequate reference samples in the relatives' database. How are these cases solved?

References: www.cmp-cyprus.org

299. TRACES OF NAZI REPRESSION IN LODZ REGION OF POLAND - GENETIC VERIFICATION OF HISTORICAL TRUTH

Jacewicz R.[1], Bąbol-Pokora K.[1], Ławrynowicz O.[2], Ossowski A.[3], Piątek J.[3], Berent J.[1]

[1] *Forensic and Medical Genetics Laboratory, Department of Forensic Medicine, Medical University of Lodz, Poland*
[2] *Archaeology Institute, University of Lodz, Poland*
[3] *Department of Forensic Medicine, Pomeranian Medical University in Szczecin, Poland*

renata.jacewicz@umed.lodz.pl

The paper presents the results of investigation of remains exhumed in places of Nazi crimes in the regions of Lodz, ie. the former training ground BRUS and the forest area LUĆMIERZ. The latter crime was the public executions of 100 Poles as revenge for the killing of 2 German policemen. The victim bodies were buried at first in the forest areas. Later they were transferred by the Germans to another place in order to remove the traces of the crime. Archeological works have led to the discovery of the original grave, which then has been emptied. A year later another place with plenty of human remains was established near the execution place. Probably it is a final burial place of Nazi victims from execution of 100 Poles in LUĆMIERZ. In order to perform verification of available documents and eyewitness accounts of the execution the genetic identification of the exhumed remains are carried out. The crucial thing is also searching for living members of the victim families in connection with the Polish Genetic Database of Totalitarianisms Victims (PBGOT).

1. Dębska E, Nowakowski PA, Jacewicz R, Bąbol-Pokora K, Prośniak A, Jędrzejczyk M, Berent J: Genetic analysis of human remains exhumed during archaeological excavations on former military training ground Brus in Lodz. Arch Med Sad Kryminol. 2013; 63(2): 99-108
2. Ławrynowicz O, Nazi Germany crimes in the forest areas of Lodz. Archeological verifications of memorials. In: Ławrynowicz O, Żelazko J,

eds. Archeology of totalitarianism. Traces of Repression 1939-1956. The Institue of National Remembrance, Commission for the Prosecution of Crimes against the Polish Nation Branch in Lodz, 2015; 65-95

3. Polish Genetic Database of Totalitarianisms Victims http://www.pbgot.pl/en

300. URINARY BLADDER SWABS AS A SOURCE OF DNA FOR HUMAN IDENTIFICATION

Brito F. C. A.[1,2], Prata D. R. B. M.[1], Martha S. F. P.[1], Bottino C. G.[1,3]

[1] Instituto de Pesquisa e Perícias em Genética Forense (IPPGF), DGPTC/PCERJ, Rio de Janeiro, Brazil
[2] Instituto de Ciências Biomédicas(IB), Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil
[3] Instituto Nacional de Metrologia, Qualidade e Tecnologia (Inmetro), Duque de Caxias, Brazil

carolina.bottino.bio@gmail.com

DNA identification provides the ability to identify and compare individuals, including the possibility to determine whether there is a genetic link between them. In catastrophic events, factors like body preservation, environmental conditions and others related to the event may impair the quality of the genetic material obtained. ISFG and INTERPOL recommend, in some cases, collecting swabs from the inner mucosa of the urinary bladder; but this kind of sample isn't routinely used. The aim of this study was to verify the viability of urinary bladder swabs as a source of DNA to identify bodies without evident signs of decomposition, by means of Forensic Genetics. Swabs from the inner mucosa of the urinary bladder of 11 corpses examined at IMLAP were collected, as well as bones and muscles of each one as reference samples. DNA was extracted by the organic method or with Chelex® resin; quantified by real-time PCR and amplified with Identifiler Plus® kit. Although Chelex® method yielded lower DNA concentrations, it was enough to obtain 7 complete and 2 partial genetic profiles (mean of 11,5 markers/profile). Complete genetic profiles (16 markers) were obtained in 100% of samples extracted by the organic method, and all of them matched the reference samples' profiles. Our results show the viability of using swabs from the inner mucosa of the urinary bladder as DNA source for human identification, because besides showing reproducible and reliable results, this type of sample allows significant reduction in time and cost required for analysis.

1. Butler JM: Fundamentals of forensic DNA typing. San Diego: Elsevier Academic Press, 500 p.
2. Hartman D, Drummer O, Eckhoff C, Scheffer JW and Stringer P: The contribution of DNA to the disaster victim identification (DVI) efforts. Forensic Science International. 2011; 205 : page 52 – page 58
3. INTERPOL: Disaster Victim Identification Guide. Available in <http://www.interpol.int/content/download/10461/74507/version/4/file/guide[1].pdf>.
4. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons, TJ, Sajantila A, Scheithauer R, Schmitter H and Schneider PM: DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI). Forensic Science International: Genetics. 2007; 1 : page 3 – page 12
5. Zietkiewicz E, Witt M, Daca P, Żebracka-Gala J, Goniewicz M, Jarzab B and Witt M: Current genetic methodologies in the identification of disaster victims and in forensic analysis. Journal of Applied Genetics. 2012; 53 : page 41 – page 60

301. ASSESSMENT OF A SIMPLE POSTMORTEM DNA SAMPLING AND TYPING METHOD FOR EFFICIENT DVI AND MISSING PERSONS IDENTIFICATION

Amory S.[1], Huel R.[1], Scott A.[2], Bilic A.[1], Mundorff A.[2], Parsons T.[1]

[1] International Commission on Missing Persons, Sarajevo, Bosnia-Herzegovina

[2] Department of Anthropology, University of Tennessee, Knoxville, USA

sylvain.amory@ic-mp.org

The ability to efficiently and rapidly identify large numbers of recently deceased persons using DNA can be a significant drain on an already overburdened forensic laboratory. Depending on the scale of the event, the laboratory will likely be required to extract DNA from a variety of post mortem sample types, family or direct reference samples and perform matching of these results in a short period of time.

A streamlined sampling protocol using disposable scalpels and sterile foam applicators has been tested as a potentially efficient and rapid way to collect postmortem samples in a wide variety of DVI contexts, including those with limited access to fixed mortuary facilities. After making an incision the foam applicator is briefly rubbed into the soft tissue and fluids present and then the swab is deposited on Whatman Indicating FTA cards.

Samples were collected from 4 bodies every 5 days for 45 days to evaluate the effect of increased post mortem interval on the quality and quantity of DNA. FTA cards were then processed using either the manufacturer's purification protocol or by extracting the DNA from 3mm punches using the QIAGEN QIAamp micro kit. DNA profiles were generated from the purified punches or DNA extracts using the PowerPlex 21 System kit using optimized cycling parameters.

This study aims at providing forensic practitioners with post mortem DNA sampling guidelines for large numbers of human remains and protocols for DNA labs allowing faster, easier and more cost effective analysis of these types of samples.

302. THE QUICKGENE SYSTEM FOR ISOLATION DNA FROM OLD SKELETAL SAMPLES

Karija Vlahović M.[1], Furač I.[1], Mašić M.[1], Kubat M.[1]

[1] DNA Laboratory, Department of Forensic Medicine and Criminology, School of Medicine University of Zagreb, Zagreb, Croatia

mkarija@mef.hr

Despite many advances in forensic applications of DNA analysis and the ability to obtain quality genetic profiles from limited or compromised forensic samples, it is still a challenge to extract a good quality DNA from old skeletal remains. In mass fatality accidents such as wars, the identification of the remains of missing persons is one of the most important preconditions to fulfill the fundamental humanitarian right of families to know the fate of their beloved. The war in Croatia (1991-1995) left more than eighteen thousand killed, imprisoned and missing persons. There is still 1600 war victims missing and twenty years after the Homeland War the teeth or bone samples are the only available material for DNA analysis. In our laboratory more than 2500 skeletal samples were processed. Since 1995 the bone extraction methods using 3-day decalcification with 0.5 M EDTA coupled with phenol, silica or magnetic based purifications were successfully applied. In an effort to speed up the DNA extraction process and reduce the starting amount of the bone powder, the full demineralization approach combined with semi-automated purification system has been tested. In order to evaluate the QuickGene technology, we analyzed 27 teeth samples from different time periods (between 20 and 123 years post mortem). Efficiency of the method was assessed by determining the amount of isolated DNA in the extracts and subsequent STR genotyping. We have successfully used the full demineralization protocol coupled with the QuickGene system to obtain full STR profiles for old human remains.

303. ROBUST AND EFFICIENT NETWORK FOR DNA RECOVERY FROM HUMAN REMAINS

Colussi A., Beltramo J., Carini G., Canutti L., Lojo M. M.

Laboratorio de Análisis Comparativo de ADN, Asesoría Pericial Departamental La Plata, Dirección General de Asesorías Periciales, Suprema Corte de Justicia de la Provincia de Buenos Aires, Argentina

agcolussi@gmail.com

The DNA extraction from human remains is one of the most important

experimental challenges, both in civil cases (Post-mortem disputed paternity) as in criminal cases. The standard operating procedures (SOPs) require many approaches due to the different features concerning the remains (age, preservation methods, kind of starting material, exposure to different environmental conditions, possible presence of inhibitors and contaminants, etc)[1, 2, 3].

The aim of this report is to present a streamlined workflow in a network which covers different types of starting samples and all possible outcomes with regard to the yield and quality of the recovered DNA. This network enables a faster and more efficient procedure for DNA typing and the confirmation of the obtained DNA Profile. More than 86% of the cases which involve the analysis of human remains were solved trough this approach. The inclusion of AutoMate Express DNA Extraction System (Applied BioSystems) BTA method in this network improved the workflow shortening its length and improving the profile quality.

1. Loreille OM, Diegoli TM, Irwin JA, Coble MD and Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Science International: Genetics I (2007) 191-195.
2. Colussi AC, Viegas M, Beltrao J and Lojo M. Assessment of the effectiveness of human remains DNA typing: Analysis of 134 cases. Forensic Science International: Genetics Supplement Series (2009).
3. Irwin JA, Just RS, Loreille OM and Parsons TJ. Characterization of a modified amplification approach for improved STR recovery from severely degraded skeletal elements. Forensic Science International: Genetics 6 (2012) 578-587.

304. DNA RECOVERY FROM A GALLSTONE IN AN ACTION FOR DAMAGES

Bozzo W.[1], Carini G.[1], Pilili J.[1], Mirson D. J.[2], Lojo M. M.[1]

[1] Laboratorio de Análisis Comparativo de ADN, [2] Laboratorio de Toxicología y Química Legal, Asesoría Pericial Departamental La Plata, Dirección General de Asesorías Periciales, Suprema Corte de Justicia de la Provincia de Buenos Aires, Argentina

mercedeslojo@hotmail.com

We analyzed three samples of unusual tissue in relation to civil action for damages by medical *mala praxis*. A dry gallstone, a gallstone immersed in formalin and a gall bladder immersed in the same preservative were submitted in order to analyze the donor identity. A blood sample of the victim was sent as reference.

The dry gallstone showed the appearance of an irregular dark brown stone. Chemical analysis revealed that it was composed mainly of cholesterol. DNA was extracted using DNA IQ™ System (Promega) and quantified by means of real time PCR. DNA samples were subjected to Identifiler® Plus (Applied Biosystems) PCR amplification. The obtained DNA fragments were run on a 3130 Genetic Analyzer (Applied Biosystems/ Hitachi) and analyzed with the software GeneMapper ID v3.2.

No profile was recovered from the samples immersed in formalin whereas the dry gallstone rendered a full DNA profile only in such samples obtained by scraping the surface. A female genetic profile that did not match the victim was obtained.

Although a gallstone is an unusual sample source, in this case the DNA analysis provided important evidence in solving the dispute.

305. CASE REPORT: DNA IDENTIFICATION OF BURNED SKELETAL REMAINS

Zgonjanin D.[1, 2], Petković S.[1, 2], Maletin M.[1, 2], Vuković R.[1, 2], Drašković D.[1, 2]

[1] Institute of Forensic Medicine, Clinical Center of Vojvodina, Novi Sad, Republic of Serbia
[2] Faculty of Medicine, University of Novi Sad, Republic of Serbia

dragana.zgonjanin@forensicns.com

For identification of skeletal remains and solving missing persons cases, the forensic application of the latest DNA technology is of utmost importance. Many failed samples during human identification were accompanied by

descriptions of challenging environmental exposures, especially burning. In our casework, most extraction attempts of skeletal remains from cases of criminal burning, with intention to destroy the body, were successful. This paper represents our approach and the results obtained during identification of burned skeletal remains.

The body of the missing fifty-two-year-old taxi driver was found burned in the trunk of a car. Due to lengthy exposure to high temperature above the tank, delivered parts of bone structures suggest that bone samples in this case will not be profiled successfully. The DNA was extracted from the powder bone using organic protocol and amplified by AmpFℓSTR® Identifiler® Plus (Applied Biosystems), AmpFℓSTR® NGM™ (Applied Biosystems) and AmpFℓSTR® Yfiler® (Applied Biosystems). Complete DNA profiles obtained using all three applied kits, were a match with the reference sample victim's son. In addition, we analyzed 5 DNA extracts, corresponding to 5 different identification cases burned body from 2009. using AmpFℓSTR® NGM™ kit. Concordant results were obtained for the STR loci between AmpFℓSTR® Identifiler® kit which was used back then and AmpFℓSTR® NGM™ which is on our disposal today, including 5 additional loci expanded ESS.

Our results have shown that simple modifications to extraction techniques can dramatically improve DNA typing success and provide conclusive, reliable profiles using different amplification kits even when working with difficult samples.

1. Hagelberg E, Sykes B and Hedges R: Ancient bone DNA amplified. Nature 1989; 342: 485.
2. Hagelberg E, Gray IC and Jeffreys AJ: Identification of the skeletal remains of a murder victim by DNA analysis. Nature. 1991; 352: 427
3. Gill P. et al. Identification of the remains of the Romanov family by DNA analysis. Nat. Genet. 1994; 6: 130
4. Cattaneo C, DiMartino S, Scali S, Craig O, Grandi M and Sokol R. Determining the human origin of fragments of burnt bone: a comparative study of histological, immunological, and DNA techniques. Forensic Sci Int. 1999; 102: 181–191.
5. Kurosaki K, Matsushita T and Ueda S. Individual DNA identification from ancient human remains. Am. J. Hum. Genet. 1993; 53: 638
6. Edson SM, Ross JP, Coble MD, Parsons TJ and Barritt SM. Naming the dead–confronting the realities of rapid identification of degraded skeletal remains. Forensic Sci Rev. 2004; 16: 63–90.

306. OPTIMIZED DNA EXTRACTION METHOD FROM SKELETAL REMAINS USING DIFFERENT TYPING METHODOLOGIES IN FORENSICS

Cavalcanti P.[1], Freitas H.[1], Silva D. A.[1], Carvalho E. F.[1]

[1] Laboratório de Diagnósticos por DNA - Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro.

dayse.a.silva@gmail.com

In the forensic field, DNA typing is usually based on STR polymorphisms. But sometimes poorly preserved human remains are everything available. Beyond the need for optimized DNA extraction methodologies, it results in other issues. When DNA is severely degraded or in small amounts, STR analysis may be challenging or even in inconclusive. In such situations, there are alternatives such as mini-STRs, STR redesigned to be more sensitive and generate smaller amplicons. Other polymorphisms as indels also have potential for human identification, because of characteristics similar to STR, and can be applied to severely degraded DNA, since generate even smaller amplicons. So, this study aimed to test an optimized, less time-expensive DNA extraction methodology for human bones combined with indels and Mini-STR, on samples with previous inconclusive results to STR. So far, the amplification efficiency of 14 samples was analyzed. The 38 INDEL ID Multiplex amplification Kit showed 51.03% efficiency, significantly higher ($p \leq 0.05$) than AmpFlSTR Minifiler™ Amplification Kit (Applied Biosystems, Foster City, Calif., USA) with 41.48%. The results showed that the alternative methods analyzed for DNA typing,

combined with the optimized DNA extraction method, are promising since in less time were able to recover meaningful information on such degraded samples. Furthermore, results demonstrated a great potential for use of 38 INDEL ID Multiplex amplification Kit on forensic human identification field.

References:

1. Butler JM: Advanced Topics in Forensic DNA Typing: Methodology. London: Academic; 2011.
2. Alvarez-Cubero MJ, Saiz M, Martinez-Gonzalez LJ, Alvarez JC, Eisenberg AJ, Budowle B, Lorente JA. Genetic identification of missing persons: DNA analysis of human remains and compromised samples. Pathobiology. 2012; 79(5):228-38.
3. Butler JM, Shen Y, McCord BR: The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 2003; 48: 1054–1064.
4. Mills, RE, Luttig, CT, Larkins, CE, Beauchamp, A, Tsui, C, Pittard, WS, Devine, SE: An initial map of insertion and deletion (INDEL) variation in the human genome. Genome Res. 2006, 16:1182–1190.
5. Pereira R, Phillips C, Alves C, Amorim A, Carracedo A, Gusmão L. A new multiplex for human identification using insertion/deletion polymorphisms. Electrophoresis. 2009; 30(21):3682-90.
6. Mulero JJ, Chang CW, Lagacé RE, Wang DY, Bas JL, McMahon TP, Hennessy LK. Development and validation of the AmpFlSTR MiniFiler PCR Amplification Kit: a MiniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA. J Forensic Sci. 2008; 53(4):838-52.
7. Silva DA, Cavalcanti P, Freitas H, Carvalho EF. High quality DNA from human remains obtained by using the Maxwell® 16 automated methodology. Forensic Science International: Genetics Supplement Series, 2013; 4(1):e248–e249.

307. COMPARISON STUDY FOR THE COMMERCIAL STR KITS ON ANCIENT HUMAN SAMPLES

Yediay F. E.[1], Allentoft M. E.[2], Simonsen B. T.[3], Hjort B. B.[3], Demirbas Y.[1], Stenderup J. T.[2], Altuncul H.[1], Hansen A. J.[2], Willerslev E.[2]

[1] Institute of Forensic Sciences, Istanbul University, Istanbul, Turkey
[2] Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark
[3] Institute of Forensic Medicine, Section of Forensic Genetics, University of Copenhagen, Copenhagen, Denmark

feylemy@gmail.com

DNA recovery from samples that undergo postmortem decay provide access to genetic information of ancient human. Multiplex STR kits are preferred for DNA identification in forensic genetics especially when new mini-STR loci have been added to these kits which allow analysis of degraded samples. In this study, 35 human bones that are taken from Van-Yoncatepe necropolis excavation were used to compare 3 STR systems (AmpFℓSTR® Identifiler®, AmpFℓSTR® Minifiler™, AmpFℓSTR® NGM SElect™), in order to determine efficiency of these kits for degraded samples and Ancient DNA.

DNA extraction from bone samples were performed by silica-based method. DNA typing was done using 3 STR kits on the samples which are genomic DNA determined by real-time PCR. 0,005-0,059 ng/µl genomic DNA was determined in 35 bones belonging to four individuals. Obtained alleles were evaluated by considering peak imbalances and rfu values. According to these evaluations, the following AmpFℓSTR® NGM SElect™, AmpFℓSTR® MiniFiler™, AmpFℓSTR® Identifiler® kits have shown multiple reliable loci. AmpFℓSTR® NGM SElect™ kit provided more genetic information in contrast with the other kits by considering the number of reliable loci.

In conclusion, STR analyses on bones that are about 3000 years old and the used method contribute greatly to molecular archeology studies and forensic sciences in terms of work on degraded samples and especially bone samples.

308. PRE-EXTRACTION REMOVAL OF PCR INHIBITORY AGENT (VIVIANITE) FOUND ON EXCAVATED BONES

Votrubova J.[1], Brzobohata H.[2], Vanek D.[1,3]

[1] Forensic DNA service, Budinova 2, 180 81 Prague 8, Czech Republic
[2] Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, Czech Republic
[3] 2nd Faculty of Medicine of Charles University, V Uvalu 84, 150 06 Prague 5, Czech Republic

Analyzing of old bone remains from archaeological sites is always challenging. This type of samples can be defined ad low quantity of degraded DNA with co-extracted inhibitors.

The collection of ten 250+ years old bone remains found in a mass grave connected with the Battle of Reichenberg, which was fought on April 21, 1757, was submitted for DNA based identification analysis. The initial tests have shown that DNA extracts contain unknown co-extracted inhibitors. The chemical analysis discovered a presence of chemical compound called vivianite ($Fe_3(PO_4)_3 \cdot 8H_2O$). Vivianite, which is often found on the fossil shells or bones in a form of deep blue coloured crystals, seems to be very strong PCR inhibitor. We have discovered that vivianite can be removed from bone samples prior to DNA extraction using a cleaning process involving sodium acid carbonate solution ($NaHCO_3$) and dialysis. The removal of vivianite from the DNA extracts enabled us to perform STR and mtDNA analysis of the bone samples in question.

309. BONE DNA TYPING USING A NEW MINIYSTR MULTIPLEX

Muniz-Orlando L. B.[1], Silva D. A.[2], Pontes I. M.[1], Carvalho E. F.[2], Filho S. A.[1]

[1] Laboratório de Diagnóstico Molecular, Universidade Federal do Amazonas, Manaus, Brazil
[2] Laboratório de Diagnóstico por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

dayse.a.silva@gmail.com

In this study, DNA samples were amplified with the multiplex MiniYSTR 09, developed for research studies of ancient and degraded forensic DNA samples. These multiplex contain nine short amplicon Y-chromosomal short tandem repeat (miniY-STR), (DYS442, DYS444, DYS445, DYS447, DYS448 and DYS461, DYS570, DYS576, DYS626). In order to investigate the efficiency of this multiplex amplification for forensic cases, DNA sample from human bone were tested. Eight male DNA samples, belonging to the DNA diagnostic laboratory of UERJ, previously extracted from remains (human bone) and amplified with commercial kits AmpFlSTR® Yfiler™, AmpFlSTR® MiniFiler™ and AmpFlSTR Identifiler®™(Thermo Fisher) were used. The result showed that four samples had full amplification using the Mini YSTR09 and four had partial amplifications, ranging from five to eight markers.The MiniYSTR loci DYS442, DYS444, and DYS461 were amplified in all samples and DYS445, DYS448 and DYS576 on seven of eight samples. Comparing the result of amplification with the commercial kits, the Mini YSTR09 had efficient and satisfactory results. Of the four samples that had full amplification with the MiniYSTR09, two samples presented only full amplification using AmpFlSTR® MiniFiler kit™. We conclude that the multiplex are highly useful for forensic practices, being able to amplify male DNA from human bone sample, thus increasing the power of discrimination of YSTRs.

1. Mameli A, Piras G, Delogu G. The successful recovery of low copy number and degraded DNA from bones exposed to seawater suitable for generating a DNA STR profile. J Forensic Sci. 2014 Mar;59(2):470-3.
2. Marjanović D, Durmić-Pasić A, Kovacević L, Avdić J, Dzehverović M, Haverić S, Ramić J, Kalamujić B, Lukić Bilela L, Skaro V, Projić P, Bajrović K, Drobnic K,Davoren J, Primorac D. Identification of skeletal remains of Communist Armed Forces victims during and after World War II: combined Y-chromosome (STR) and MiniSTR approach. Croat Med J. 2009

Jun;50(3):296-304.

310. A COMPARATIVE STUDY BETWEEN MUSCLE, CARTILAGE AND SWAB FROM INSIDE THE URINARY BLADDER SAMPLES FOR DNA TYPING OF SEVERELY BURNT BODIES IN DISASTER VICTIM IDENTIFICATION (DVI)

Ferreira S. T. G.[1, 2], Paula K. A.[1], Nogueira R. C.[3], Oliveira E. A.[3], Moraes A. V.[1]

[1] Forensic DNA Research Institute, Federal District Civil Police, Brasília, Brazil
[2] National Secretariat of Public Security, Ministry of Justice, Brasília, Brazil
[3] Institute of Legal Medicine, Federal District Civil Police, Brasília, Brazil

samueldoc83@yahoo.com.br

The choice of samples for DNA typing of burnt bodies plays an important role in disaster victim identification (DVI). In this study, we compared DNA yields and STRs profiles of deep red muscle, cartilage from joints and swab from inside the urinary bladder samples collected from three severely burnt female bodies due to a car accident on a highway in Brazil. We collected the three kinds of samples from the three bodies and compared each other. As the bodies were severely burnt, it was not possible to perform identification by dental, fingerpriting or anthropological analysis. The identification was performed by DNA testing. We used direct reference samples (tooth brushes) and family reference samples (mothers, brothers and son) for the comparison with the post mortem samples and identification of the victims. We used modified organic phenol-chloroform for DNA extraction, Plexor HY Quantification Kit (Promega Corporation) to quantify the DNA in a 7500 Real-Time PCR System (Applied Biosystems) and PowerPlex Fusion Kit (Promega Corporation) for the amplification. PCR products were analyzed on an 3130xl Genetic Analyzer (Applied Biosystems). The amount of DNA recovered from each sample and the quality of the STRs profiles obtained demonstrated that deep red muscle, cartilage and swab from inside the urinary bladder may be excellent sources of samples for DNA typing of severely burnt bodies in disaster victim identification (DVI).

311. EXAMPLES OF EXCEPTIONAL HUMAN IDENTIFICATIONS DONE BY DEPARTMENT OF FORENSIC GENETICS IN SZCZECIN, POLAND

Zielińska G.[1], Ossowski A.[1], Kuś M.[1]

Department of Forensic Genetic, Pomeranian Medical University in Szczecin, Poland

forensicmedicine1980@gmail.com

The research shows example of three identification processes, each with different level of difficulty. Even though all of them used methods typical for forensic genetics, each had to be treated in a very special way.

First case concerns determination of maternity and paternity of four newborns. Infants were found dead, buried in a garden in a plastic bags. The remains were skeletonized with mortuary wax present. Femur from each newborn was used for a genetic testing. Second case concerns a drowning of a homeless man, whose body was found by a fisherman. Although the ID was present, the family did not affirm man's identity. Genetic material coming from half siblings (two sisters and two brothers) was used as a reference. For the final identification all the possible analysis had to be done: autosomal STR, X-STR, Y-STR and mitochondrial DNA. The last case concerns a murder of an elderly man. He was killed and then buried with the use of roofting felt. It caused complete reduction to ashes. Meticulous anthropological analysis helped with selecting single bone fragment proper for a genetic testing. The obtained profile was used for the identification and solving the crime.

The research shows that genetic testing is very useful in a final identification of unknown bodies. In spite of the impact of a various factors of degradation, reliable and repeatable results can be obtained.

312. GENETIC IDENTIFICATION OF BURNED CORPSES AS A PART OF DISASTER VICTIM IDENTIFICATION EFFORT

Ricci U.[1], Carboni I.[1], Iozzi S.[1], Nutini A. L.[1], Contini E.[1], Torricelli F.[1], Focardi M.[2], Pinchi V.[2], Norelli G. A.[2]

[1] Diagnostic Genetics Unit, AOU Careggi, Florence, Italy
[2] Department of Health Sciences, Forensic Sciences Section, University of Florence

ricciu@aou-careggi.toscana.it

The Italian town of Prato, just north of Florence, has been a centre for the Italian garment trade since the 12th century and now hosts a number of Chinese-run textile factories producing garments for export across Europe. In December 2013 a fire broke out one of this factory, where eleven Chinese workers were employed. After the fire was extinguished, remains of seven people were collected by rescuers. In order to identify the victims and to establish the cause of death a pool of forensic experts (including pathologists, odontologists, toxicologists and geneticists) worked together to identify the victims, in accordance with disaster victim identification (DVI) protocol. In this case direct genetic comparisons was not possible between the DNA profile of victims and DNA profile generated from reference samples. Thus, seven related subjects' DNA were acquired for indirect comparisons with the victims. Just a relative to each of the missing persons (children, parents, brothers or sisters) was available for the comparison. The contribution of forensic genetics in this case has proven essential in the identification of some bodies, especially of body parts (arms and hands) that otherwise would not be possible to attribute to each victim. In particular, here we show the protocol adopted that included the use of a wide battery of markers STRs, Y-STR and biostatistics evaluation of different assumptions about the identification of these persons. Finally, but not least, the collaboration among all professionals involved in the identification via the DVI protocol proved to be the most important thing in this bloody episode.

313. DNA EXTRACTION AND PURIFICATION FROM ARCHIVAL MATERIAL: REVISITING THE STANDARD OPERATING PROCEDURE

Netto M. S. C.[1], Alves A. L. C.[1], Carvalho E. F.[2], Garrido R. G.[1], Silva D. A.[2]

[1] Instituto de Pesquisa e Perícias em Genética Forense, DGPTC, PCERJ, Rio de Janeiro, Brasil
[2] Laboratório de Diagnósticos por DNA, DECOL, IBRAG, Universidade do Estado do Rio de Janeiro, Brasil

dayse.a.silva@gmail.com

Forensic evidence from tissues embedded in paraffin archived for long periods and safeguarded on Pathology and Genetics Institute are, in some cases, the only register available of the collected sample. However, recovering DNA from this material can be challenging although there are many protocols specific for forensic context. The aim of this study is to evaluate methods of extraction and purification of DNA, using biological archival materials, in order to determine a protocol that is easy use and efficiency to adapt to the Institute of Research and Expertise in Forensic Genetics (IPPGF). Twenty two samples from cadaver tissues collected from 1983 to 2011 and included in paraffin blocks were submitted to two pre-treatments: deparaffination using xylene and heating in microwave. After that, four methods were used for DNA extraction: Phenol-chloroform-isoamyl alcohol (PCI), Chelex 100®, Purification on membranes (NucleoSpin) and the Resin purification method (DNA IQ).

The quality and quantity of the DNA extracted was compared using qPCR. Additionaly, PCR reactions (STR amplifications) were performed using the MiniFiler™ Kit. The methodology that presented better DNA recovery in relation to the age of the tissues and also for the different tissues embedded in paraffin was PCI. The methodologies that presented more loci amplified were PCI and Nucleospin, followed by IQ DNA and Chelex. These results reinforce and validate the maintenance of Standard Operating Procedure based on PCI used in IPPGF.

1. Barea, J. A.; Pardini, M. I. M. C.; Gushiken, T. Extraction of DNA from archival material, and few sources: review and notes on possible applications. NewsLab, v. 63, p. 96-114, 2004.
2. Budeowle, B. et al. Simple Protocols for Typing Forensic Biological Evidence: Chemiluminescent Detection for Human DNA Quantitation and RFLP Analyses and Manual Typing of PCR Amplified Polymorphisms. Electrophoresis, v. 16, n. 9, p.1559-1567, 1995.
3. Huijsmans, C. J. et al. Comparative analysis of four methods to extract DNA from paraffin-embedded tissues: effect on downstream molecular applications. BMC Res. Notes, n. 3, p. 239, 2010.

314. A MATERNITY CASE WITH HUMAN REMAINS FROM A XIII-XIV CENTURY BURIAL AT UCEDA, GUADALAJARA, CENTRAL SPAIN

Gomes C.[1], Palomo-Díez S.[1], Dorado-Fernandéz E.[2], Ruiz-Mediavilla E.[3], Magaña-Loarte C.[2], Ramírez-González I.[4], López-Parra A.[1], Baeza-Richer C.[1], Gibaja J.[5], Arroyo-Pardo E.[1]

[1] Forensic and Population Genetics Group, Department of Toxicology and Legal Medicine from Complutense University, Madrid, Spain
[2] Laboratory of Forensic Anthropology, Forensic Anatomical Institute, Madrid, Spain
[3] School of Legal Medicine, Department of Toxicology and Legal Medicine from Complutense University, Madrid, Spain
[4] Archaeologist. Spain
[5] Department of Archaeology and Antropology (IMF-CSIC), Barcelona,Spain

clopes01@ucm.es

In a High Medieval Age cemetery, dated from the XIII-XIV century (Uceda, Guadalajara, Central Spain), two bodies were found, buried in a curious position. One of the bodies, an adult, had close to its abdominal area a small number of little bones. It was not clear if it could have been a pregnant woman or, otherwise, two separated burials, at different times.

Anthropological experts confirmed that the second individual should be a fetus, being absolutely impossible to determine the sex. Furthermore, the adult was appointed as a woman, with some uncertainty.

Concerning to the condition of the samples, the adult ones were preserved, obeying to the authenticity criteria to select evidences for a critical DNA analysis (1). But the samples belonging to the second individual were very delicate and fragile, complicating the sampling work.

A genetic study will be carried out to find if there is any biological bond between the individuals, as well as, their biological sex. The analysis procedure had to be somewhat modified due to the sensitivity of the second individual samples.

So far, our preliminary results reveal that, if both individuals are not linked by maternal kinship, they must be, at least, relatives by maternal side, since they share the same maternal lineage.

Conclusions reached in the present study can help in mass disasters cases. In such situations, it is crucial to determine kinships between samples, despite their advanced state of degradation, which makes the improvement of this procedure a crucial point in forensic genetics.

1. Pääbo S, Poinar H,[1]Serre D, Jaenicke-Després V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, and Hofreiter M. 2004. Genetic Analyses from Ancient Dna. Annual Review of Genetics. DOI: 10.1146/annurev.genet.37.110801.143214

315. RESTORED TEETH CAN BE USED AS SAMPLES FOR GENOTYPING?

Corte-Real A.[1], Vieira D. N.[1] , Corte Real F.[1], Anjos M. J.[2]

[1] Faculty of Medicine, University of Coimbra, Portugal
[2] Central Branch, National Institute of Legal Medicine and Forensic Science, Coimbra, Portugal

a.corte.real4@gmail.com

Teeth with direct restorations have been excluded as samples for genetic

analysis. The aim of this study is to warn that such samples should not be overlooked for a successful genotyping.

Six restored dental pieces were randomly selected from forensic situations of INMLCF, IP, and two portions have been selected: it was analysed the dentin of adjacent portions of the restoration area in itself and the remaining root dentin. DNA extraction was executed using the PrepFiler™BTA kit according to the manufacturer's instructions and its quantification was realized with the Quantifiler™Duo kit (both from Applied Biosystems). The results, associated with this study were analyzed with SPSS Statistics™ 19th version.

In one sample it was just possible to determine autosomal profile from DNA of the DS portion, while the corresponding remaining root portion allowed the characterization of a mitochondrial DNA profile. In the other portions DS quantification values were comparatively higher than those of remaining portion.

These results may be related to a tertiary dentin formation, restorative and disorganized. This dentin leads to the entrapment of cellular elements in its mineralized matrix, forming a barrier between the external (oral cavity) and internal (pulp cavity) spaces.The use of a sample with dentin tissue adjacent to a restoration area, covering the entire portion up to the pulp cavity, in order to include the potential restorative tertiary dentin, proved to be a good sample for genetic analysis, optimizing the quality of the sample volume.

1. Ballantyne KN, Van-Oorschot RAH, Motchell RJ. Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples. For Sci Int 2007;166; 16635-41
2. Burger, J et al. DNA preservation: A microsatellite-DNA study on ancient skeletal remains. "Electrophoresis 1999; 20; 1722-1728
3. Dixon LA et al. Analysis of artificially degraded DNA using STRs and SNPs – results of a collaborative European (EDNAP) exercise. For Sci Int 2006;164;33-44
4. Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. For Sci Int 2000; 112; 17–40
5. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int: Genetics 2007;1;191-195
6. Butler JM, Shen Y, McCord BR. The development of reduced size STR amplicons as tools for analysis of degraded DNA. J For Sci 2003; 48,1054–64
7. Welch LA, Gill P, Phillips C et al.European Network of Forensic Science Institutes (ENFSI): Evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers. Forensic Sci Int: Genetics 2012; 6:819-826.
8. Garant PR. Chapter 2 – Dentin. In Dickson A, editor. Oral cells and tissues. Chicago: Quintessence Publishing Co, Inc 2003;25-52.
9. Corte-Real A, Anjos MJ, Vieira DN, Gamero JJ. The tooth for molecular analysis and identification: a forensic approach. J Forensic Odonto-stomatol 2012; 30; 22-28
10. Corte-Real A, Anjos MJ, Serra A, Carvalho M, Gamero J, Vieira DN. Tooth portion profile in criminology. Forensic Sci Int: Genetics 2011;e433–e434

316. A COMBINED DNA EXTRACTION METHOD FOR BONES AND TEETH

Desmyter S.[1], De Cock G.[1], Moulin S.[1], Noël F.[1]

[1] *Nationaal Instituut voor Criminologie en Criminalistiek, Brussels, Belgium*

stijn.desmyter@just.fgov.be

Over the last decade the DNA extraction from bone material underwent two important modifications: a total demineralization as a first step in the protocol and the classical organic extraction with phenol/chloroform being replaced by silica-based purification methods.

In our laboratory, the DNA IQ™ Casework Sample Kit for Maxwell®16

(Promega) has been progressively validated for routine analysis of biological traces like saliva, blood, hairs and also bones/teeth. This paramagnetic silica-coated beads extraction kit results in a better removal of PCR inhibitors and enables automation.

Despite the fact that total demineralization followed by purification with paramagnetic beads resulted in successful genetic identification in the majority of our case work samples, the bones of three human remains failed to be STR-profiled. Two of them had been buried for several years before extraction and the third had been recovered from sea water.

In order to obtain DNA-profiles for these three human remains, bone fragments were subjected to an alternative extraction method in which the purification with Maxwell®16 was preceded by an organic extraction. This combined phenol/Maxwell®16 method did yield sufficient amounts of DNA for complete STR-profiling by ESIPro/ESX® (Promega) of all three human remains. In addition, a series of bones from three other human remains that had been buried for over 30 years were successfully analysed by this combined method.

This phenol/Maxwell®16 method was further tested on other biological samples with a high volume matrix that showed poor or no DNA recovery after single Maxwell®16 purification, in particular fly maggots and degraded organ tissue.

317. A PRIMARY INVESTIGATION ON SNPS ASSOCIATED WITH EYELID TRAITS OF CHINESE HAN ADULTS

Jin B.[1,2], Zhu J.[1], Wang H.[3], Chen D.[3], Su Q.[1], Wang L.[1], Liang W.[1,*], Zhang L.[1,*]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Medicine, North Sichuan Medical College, Nanchong 637000, Sichuan, China
[3] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081,Sichuan, China

*Corresponding author: liangweibo@scu.edu.cn

Predicting group-specific physical traits using genetic markers is an important subfield in forensic individual identification. Commercial kits including SNPs used to predict hair and iris color with necessary high degrees of accuracy have been developed and applied in legal practice for several years. But the kits are valid only for person with European geographic origin. Few SNPs associated with specific physical traits of Asian have been found till now.

Being one of the typical oriental externally visible characteristics, single/double eyelid and epicanthus are race specific "trademark" physical traits with relative high heritability in Asian population. Six SNPs have been screened out from genes associated with eyelid development through target capture Massively Parallel Sequencing (NGS) and typed with TaqMan® probes using ABI 7500 Real-Time PCR System in preliminary experiment. Results demonstrated that SNP rs2277404 on ABCC9 gene had significantly different distribution of the genotype frequency between population with single or double eyelid ($p < 0.05$), SNP rs60650442 on FOXL2 gene had significantly different genotype and allele frequency distribution between people with or without epicanthus ($p < 0.05$), which means eyelid traits are of promising expectation for accurate DNA prediction.

Subsequently, we plan to select SNPs highly associated with eyelid traits in Chinese Han population with large sample size and then validate the identification power of screened SNPs for predicting such traits of unknown people. We aim to develop genetic markers for individual identification of specific physical traits to be useful in forensic applications in Asian.

318. THE CASE OF 2 SIBLINGS THAT IDENTIFIED NOT ONLY BY DNA PROFILING

Maeda K.[1], Murakami C.[1], Irie W.[1], Sasaki C.[1], Oishi M.[1], Nakamaru N.[1], Nakamura S.[1], Kurihara K.[1]

[1] Department of Legal Medicine, Kitasato University School of Medicine, Sagamihara, Japan

maedak@med.kitasato-u.ac.jp

Short tandem repeat (STR) profile is widely used as a standard technique for identification of individuals because of its high power of discrimination. However, the existence of the kinship would be necessary to identify the missing person. We present a rare case of 2 charred remains that could not identify only by using DNA profiling.

Two charred remains were found from fire in the house where 2 brothers suppose to be lived. The elder brother was in his eighties and younger brother was in his sixties. But we could not tell one from other. Since they had no kinship except their sister, DNA profiling was performed with the sister who is alive. As a result, the calculation of the probability of sibling was sufficient to determine the 2 remains as brothers. Though, it was still impossible to distinguish which remains would be the elder or younger brother. Fortunately, younger brothers dental chart was obtained by the police search and the dental identification settled one remains as younger brother.

In this case, the dental information helped to identify the individuals, not all cases could be the same situation. Meanwhile, there was an age difference between the 2 brothers. Recent study demonstrated that several molecular methods could used to estimate human age. However, those biomarkers have relatively low precision and practical limitations, it is still hard for practical use. Establishment of the procedure would be necessary in order to help the human identification in such situation as an accident or mass disaster.

319. A GRAVE IN MY GARDEN. GENETIC IDENTIFICATION OF SPANISH CIVIL WAR VICTIMS

Nuñez C.[1], Baeta M.[1], Palencia-Madrid L.[1], Herrasti L.[2], Etxeberria F.[2], de Pancorbo M. M.[1]

[1] BIOMICs Research Group, Lascaray Research Center, University of the Basque Country UPV/EHU. Avda. Miguel de Unamuno, 3. 01006 Vitoria-Gasteiz (Spain)
[2] Departamento de Antropología. Sociedad de Ciencias Aranzadi. Alto de Zorroaga. 20014 Donostia-San Sebastián (Spain).

marianpancorbo@gmail.com

We report the genetic study of 13 victims of the Spanish Civil War buried in two mass graves in Espinosa de los Monteros (Burgos, Spain). The grave with the highest number of individuals (Grave 1; N=9) was found in the garden of a private house of the village. The other grave (Grave 2) was only 200 m away and the remains of 4 women were unexpectedly found. Skeletal remains, mainly teeth, were better preserved in Grave 1 than 2. Mean DNA extraction yields were 0.277 and 0.045 ng/µl, respectively. In order to carry out the genetic identification of the remains, buccal swabs from 11 family relatives were available. The choice of genetic markers analyzed relied on the different levels of biological kinship to be elucidated. For 75% of the post-mortem samples, informative autosomal STR profiles (≥12 STRs) were obtained. Y chromosome STRs, as well as mitochondrial DNA control region, were also analyzed in order to study paternal and maternal lineages, respectively. Besides, X chromosome STRs were typed in one case to verify a paternal grandmother-granddaughter relationship. We successfully identified 9 of 13 individuals buried in the two mass graves of Espinosa de los Monteros, 6 in Grave 1 and 3 in Grave2. The search of further relatives' samples would be of interest to perform more comparative analyses, and eventually do not leave any of the victims without a name.

320. STUDY OF MEDIEVAL CRITICAL SAMPLES - A GENETIC APPROACH TO THE STUDY OF THE MUDEJAR COMMUNITY

Gomes C.[1], Magaña-Loarte C.[2], Dorado-Fernandéz E.[2], Ruiz-Mediavilla E.[3], Ramírez-González I.[4], Palomo-Díez S.[1], López-Parra A.[1], Baeza-Richer C.[1], Gibaja J.[5], Arroyo-Pardo E.[1]

[1] Forensic and Population Genetics Group, Department of Toxicology and Legal Medicine, Complutense University, Madrid, Spain
[2] Laboratory of Forensic Anthropology, Forensic Anatomical Institute, Madrid, Spain
[3] School of Legal Medicine, Department of Toxicology and Legal Medicine,

Complutense University, Madrid, Spain
[4] Archaeologist. G3A, Universidad Europea, Spain
[5] Department of Archaeology and Antropology (IMF-CSIC), Barcelona,Spain

clopes01@ucm.es

On their arrival to the Iberian Peninsula, the North African and Berbers Muslims encountered no resistance, but a land with some political trouble between Christians, Godos and the Jewish people, which facilitated somehow the absorption of the new invaders ideas. In on hand, there is the idea that military invasion changed by force daily habits, religious traditions and beliefs: the "Al-Ândalus" region. However, in the other hand, there are evidences of a peaceful co-existence between Muslims and the "Iberians", as well as, a voluntary absorption of the Islamic culture. . Thus, Muslims who lived under Christian rule formed the "Mudejar" community and its biogeographical origin remains still unclear.

Considering the wide geographical extend and duration of the Muslim occupation, it is reasonable to suppose that the Islamic permanency had an significant impact on the Iberian gene-pool.

In Uceda, (Guadalajara, Spain), 70 medieval bodies were found buried in a Mudejar cemetery. According to anthropologists' experts, Muslim burial was observed, including the body orientation to Mecca, and all led to believe that the individuals had an Islamic origin. So, we are carrying out a genetic study in order to verify if their mitochondrial lineage is coincident with the characteristic haplogroups described for peoples from the actual Islamic geographical areas.

The sampling process concerned two teeth per individual, obeying to the criteria to select evidences for critical DNA analysis. The samples were directly exposed to the floor conditions, and, in some cases, were externally damaged, requiring some adaptations in the analysis process.

Our preliminary results seem to indicate that the individuals did not have North-African origin, since they revealed, so far, European haplogroups, indicating a possible religious conversion.

From the forensic perspective, this is an important study since we adapted the technique in order to obtain reliable and replicable data from critical samples that were buried and directly exposed to the floor conditions. Historically, these results could be quite challenging because ancient DNA technology can be useful to shed light into the genetically history of the Iberian Peninsula.

321. DNA EXTRACTION FROM TEETH LEFT EXPOSED TO THE ELEMENTS FOR MORE THAN 60 YEARS USING SILICA MEMBRANE COLUMN

Tsukada K., Harayama Y.

Department of Forensic Medicine,
Criminal Investigation Laboratory, Nagano Pref. Police H.Q. Japan

tuk-lab@mx1.avis.ne.jp

Abstract

DNA typing is relatively easy to perform with specimens such as blood, saliva, and semen. The same may be said of DNA typing of teeth or bone. However, it is often difficult to perform DNA typing of teeth or bone discovered in the soil in Japan, due to the acidic soil and high humidity.

In this study, we performed DNA extraction and DNA typing from four tooth present in the ground for more than 60 years.

Materials and methods

The teeth was pulverized using a multi-beads shocker. We performed DNA extraction by the AP method (Buffer ATL + Proteinase K), AEP method (Buffer ATL + 0.5 M EDTA + Proteinase K), and decalAP method (BufferATL + Proteinase K after decalcification). DNA was extracted using the QIAamp DNA Investigator kit; PCR amplification was performed using AmpFlSTR Identifiler Plus kit; electrophoresis was performed using 3130xl Genetic Analyzer.

Results

The results indicated DNA extraction by the AEP method was most efficient, while DNA extraction by the decalAP method was least efficient.

322. NONDESTRUCTIVE EXTRACTION DNA METHOD FROM BONES OR TEETH, TRUE OR FALSE?

Gomes C.[1], Palomo-Díez S.[1], Roig J.[2], López-Parra A.[1], Baeza-Richer C.[1], Esparza A.[3], Gibaja J.[4], Arroyo-Pardo E.[1]

[1] Forensic and Population Genetics Group, Department of Toxicology and Legal Medicine, Complutense University, Madrid, Spain
[2] Archaeologist. Arqueologia y Patrimoni Arrago sl. Universidad del País Vasco (UPV/EHU) Barcelona, Spain
[3] Department of Prehistory, Ancient history and Archaeology from Salamanca´s University, Salamanca, Spain
[4] Department of Archaeology and Antropology (IMF-CSIC), Barcelona, Spain

clopes01@ucm.es

Gomes C and Palomo-Díez S contributed equally to this study

One of the most challenging points for ancient DNA studies on human remains is that analyzing procedures involve at least partial destruction of the samples. In fact, most of the times, it signifies the destruction of the entire sample, and this is what prevents museums, anthropologists or archaeologists from giving samples for genetic investigation.

Usually, the samples employed are unique and irreplaceable, being interesting to find a way to analyze without destroying them. There have been several attempts to reduce the samples damage [1, 2]. However the results have not always been satisfactory. On one hand, the main problem of these methods is usually associated with the achievement of worse performance of the procedure, but on the other hand, it must be taken into account if the sample preservation after nondestructive process is total or partial.

In this study, it was carried out the evaluation of a nondestructive extraction DNA protocol based on the Rohland and Hofreiter (2007) [3, 4, 5]. It was performed the DNA extraction from 10 Neolithic samples (teeth), from 5 archaeological individuals buried in Can Gambús (Sabadell, Spain) and 8 pre Bell Beaker period samples (bones and teeth), from 4 individuals buried in Los Cercados (Valladolid, Spain).

The first sample of each individual was analyzed by the destructive protocol [3, 4, 5] and the second one by the nondestructive procedure. Along this work, it was discussed the efficiency of the nondestructive protocol, valuing the obtained result, and the evidences state of preservation after the whole procedure.

1. Hofreiter, M. Nondestructive DNA extraction from museum specimens. Methods Mol Biol. 2012;840:93-100.
2. Molhandesan, E., Prost, S., Hofreiter, M. 2012. Case estudy: Using a nondestructive DNA extraction method to generate mtDNA sequences from historical chimpanzee specimens. Methods Mol Biol. 2012; 840: 101-10. doi: 10.1007/978-1-61779-516-9_14
3. Rohland, N. Y Hofreiter, M. 2007a. Ancient DNA extraction from bones an teeth. Nat. Protocols 2: 1756-1762
4. Rohland, N. Y Hofreiter, M. 2007b. comparison and optimization of ancient DNa extraction. Bio Techniques 42: 343-352.
5. Rohland, N., Siedel, H. and Hofreiter, M. 2009. A rapid column - based ancient DNA extraction method for increased sample throughput. Molecular Ecology. Resources. Doi: 10.1111/j.1755-0998.1009.02824.

323. POPULATION GENETICS OF TURKISH CYPRIOTS FROM CYPRUS: FORENSIC AND ANTHROPOLOGICAL IMPLICATIONS

Gurkan C.[a], Demirdov D. K.[a], Sevay H.[b]

[a] Turkish Cypriot DNA Laboratory, Nicosia, North Cyprus
[b] Department of Information Systems Engineering, Near East University, Nicosia, North Cyprus

cemal.gurkan@gmail.com

Turkish Cypriot DNA Laboratory (TCDL) operates under the Turkish Cypriot Member Office of the Committee on Missing Persons in Cyprus (CMP). TCDL

contributes to the UN-led CMP Project on "Exhumation, Identification and Return of Remains of Missing Persons", and as such, it also plays a pioneering role in the establishment of forensic genetic services in North Cyprus. As the first task, a family reference sample (FRS) bank comprising 1,100+ samples from the relatives of the Turkish Cypriot (TC) missing persons (MiPs) from the 1963/64 and 1974 era was established. While initial DNA profiling of these FRS samples were conducted elsewhere, TCDL assumed full responsibility for all DNA profiling of TC FRSs since 2012. So far, ~400 such FRS profiles have been reported by TCDL, which were used for familal DNA searching/matching with the profiles of skeletal remains obtained at international laboratories such as ICMP and Bode Technology. TCDL also compiles population data for statistical evaluations of MiP identifications. To this end, more than 500 other samples - none of which belonging to MiP relatives - have been analyzed by autosomal and Y-chromosomal STR typing methods and the results were recently published 1,2.

1. Gurkan C, Demirdov DK, Yamaci RF and Sevay H: Population genetic data for 15 autosomal STR markers in Turkish Cypriots from Cyprus. Forensic Sci Int Genet. 2015; 14: e1-e3.
2. Terali K, Zorlu T, Bulbul O and Gurkan C: Population genetics of 17 Y-STR markers in Turkish Cypriots from Cyprus. Forensic Sci Int Genet. 2014; 10: e1-e3.

324. SNPFORID 52PLEX IN CASEWORK SAMPLES: "CRACKING" BONES AND OTHER DIFFICULT SAMPLES

Dario P.[1,2,3,4], Oliveira A. R.[1,2,4], Ribeiro T.[1,3], Porto M. J.[1,3], Costa Santos J.[1,3,5], Dias D.[2,4], Corte Real F.[1,3,6]

[1] *National Institute of Legal Medicine and Forensic Sciences, Portugal*
[2] *Faculty of Science, University of Lisbon, Lisbon, Portugal*
[3] *CENCIFOR - Forensic Sciences Centre, Coimbra, Portugal*
[4] *CESAM - Centre for Environmental and Marine Studies, Lisbon, Portugal*
[5] *Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[6] *Faculty of Medicine, University of Coimbra, Coimbra, Portugal*

mariajoao.porto@dcinml.mj.pt

Casework samples can present difficulties to forensic scientists in criminal and identification investigations. Some challenging samples like bones, teeth and crime samples, often contains little DNA which can even be degraded. In these cases STR profiles obtained after PCR amplification are many times incomplete or null. This is partly due to the size of the STRs commonly used in forensic analysis and in order to bypass this problem other analysis strategies have been developed in the past: mini-STRs and biallelic markers, such as InDels and SNPs. Even though each marker type has its vantages and advantages, SNPs benefit from having the smaller amplification products and its analysis can be realized analyzing simultaneously 52 markers in SNP*for*ID 52-plex, providing good results as reported by other authors. Taking this in consideration we compared the amplification success of 53 real casework samples from our casuistic consisting of bones, teeth and others using 52-plex and Identifier® Amplification PCR kit. The mean amplification success rate by loci was of 73% and 43% respectively and 16 out of 36 samples in which STR profiles were not obtained or in which these were poor, generated complete or almost complete SNP profiles. We conclude that 52-plex can be a valuable tool in the analysis of different types of challenging forensic samples when STRs fail to provide the necessary genetic information for identification.

1. Sanchez JJ, Phillips C, Borsting C, et al. A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis. 2006; 27:1713–1724.
2. Musgrave-Brown E, Ballard D, Balogh K, et al. Forensic validation of the SNPforID 52-plex assay. Forensic Sci Int Genet. 2007; 1:186–190.
3. Romanini C, Catelli ML, Borosky A, et al. Typing short amplicon binary polymorphisms: supplementary SNP and Indel genetic information in the analysis of highly degraded skeletal remains. Forensic Sci Int Genet. 2012; 6:469–76.

4. Alimat S, Hadi S, Goodwin W. SNP genotyping of forensic casework samples using the 52 SNPforID markers. Forensic Sci Int Genet Suppl Ser. 2013. 4:e178–e179.

325. THE COLLABORATIVE EXERCISE CONCEPT ON DNA TYPING OF BONE SAMPLES

Vanek D.[1,2], Budowle B.[3,4], Votrubova J.[1]

[1] *Forensic DNA Service, Prague, Czech Republic*
[2] *Charles University in Prague, 2nd Faculty of Medicine, Prague, Czech Republic*
[3] *Institute of Applied Genetics, University of North Texas Health Science Center, TX 76107, USA*
[4] *Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia*

A collaborative exercise on DNA Typing of bone samples was organized in 2012. The aim of the study was to test the performance among different laboratories of DNA analysis from relatively old bone samples. The bone samples (provided by the National Museum and the Institute of Archaelogy Prague, Czech Republic) came from archaeological excavations and were approximately 150 and 400 years old. The method of genetic characterization (autosomal, gonosomal, mitochondrial markers) was solely at the discretion of the participating laboratory.. The results indicated that although different extraction and amplification strategies were employed, concordant results were obtained from the relatively intact 150 year old bone sample. Typing was more problematic with the analysis of the lower quality 400 year old bone sample. The results clearly show that the concept of the collaborative exercise (CE) should change in order to gather more information on validity and reliability of typing challenged bone samples. The concept of future collaborative exercises on bone samples could provide insight on typing results from extraction-borne variations by sending participating labs DNA extracted from aged bone samples. Focused studies may help to better identify the root cause of particular DNA typing limitations (e.g. by the extraction method) and anomalies. The future CE also could address the cleaning and grinding phase and/or the removal of humic acid inhibitors and modern DNA contaminants. Another improvement of the CE concept would be the inclusion of massive parallel sequencing during verification of the sample by the organizing laboratory.

326. LOOKING FOR A RELIABLE CRITERIA FOR THE ESTABLISHMENT OF SOLID STR PROFILES USING ANCIENT CRITICAL SAMPLES FROM 3000 TO 4000 YEARS AGO

Palomo-Díez S.[1], Gomes C.[1], López-Parra A.[1], Baeza-Richer C.[1], Esparza-Arroyo A.[2], Arroyo-Pardo E.[1]

[1] *Forensic and Population genetics group, Department of Toxicology and Legal Medicine from Complutense University of Madrid*
[2] *Department of prehistory, ancient history and archaeology from Salamanca´s University*

clopes01@ucm.es

The establishment of a consensus STR profile from partial STR profiles of Low Template DNA (LTD) samples is a well-known problem [1, 2, 3, 4].

Through this research different criteria has been proposed and evaluated for the interpretation of partial STR profiles obtained from critical samples trying to establish the most reliable one. Precisely, 84 samples of 42 individuals (2 samples from each one) have been employed. Mentioned samples come from 6 different Chalcolithic and Bronze Age sites (3000 to 4000 years old). Initially, 5 different criteria to select peaks and create STR profiles have been established, taking into account information like the height of the peaks measured in rfus and the reproducibility of results by the amplification of the same sample and between the two samples of each individual. This research discuss about the importance of obtaining reproducible results [4].

Secondly, there has been carried out the kinship analysis between the individuals buried together to determine possible familiar relationships. In this second part there has been taken into account also the analysis of other genetic

markers, like the mitochondrial DNA, to consolidate the obtained results by STR analysis. In this way, it is possible to go in depth about which criteria could generate false kinship results.

Along this work we have tried to evaluate which is the best criterion to establish STR profiles from LTD samples, with the principal premise that the best criterion is the one that provides more quantity of information without producing false positives.

1. Gill, P., Gusmao, L, Haned, H., Mayr, W.R., Morling, N., Parson, W., Prieto, L., Prinz, M., Schneider, H., Schneider, P.M., WEIR, B.S. 2012. DNA commission of the international society of Forensic Genetics: Recommendatios on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods. Forensic Science International: Genetics. N. 6. 679-688.
2. Gittelson, S., Biedermann, A., Bozza, S., Taroni, F. 2014. Decision analysis for the genotype designation in low-template-DNA profiles. Forensic Science International: Genetics. N.9. 118-133.
3. Grisedale, K.S. and van Daal, A. 2012. Comparision of STR profiling from low template DNA extracts with and without the consensus profiling method. Investigative Genetics, 3:14.
4. Kokshoorn, B., and Blankers, B.J. 2012. Response to Grisedale and Van Daal: comparison of STR profiling from low template DNA extracts with and without the consensus profiling method. Investigative Genetics, 4:1.

327. KINSHIP ANALYSIS IN MASS GRAVES: EVALUATION OF THE BLIND SEARCH TOOL OF THE FAMILIAS 3.0 SOFTWARE IN CRITICAL SAMPLES

Palomo-Díez S.[1], López-Parra A.[1], Gomes C.[1], Baeza-Richer C.[1], Esparza-Arroyo A.[2], Arroyo-Pardo E.[1]

[1] Forensic and Population Genetics Group, Department of Toxicology and Legal Medicine from Complutense University of Madrid

[2] Department of prehistory, ancient history and archaeology from Salamanca´s University

clopes01@ucm.es

The individualization of skeletal remains from mass graves, as well as the kinship analysis between individuals buried together, involves a problem that is further increased when the remains are degraded because of the environmental conditions or the antiquity of the burial. Hence, frequently we must face up to LTD samples.

To deal with these kind of problems, where the kinship degree between the individuals is completely unknown, *Kling et* al. (2014) have developed an interesting tool called *Blind search tool* in the context of the last *Familias* 3.0 software [1]. This tool is capable to find randomly kinship relationships between multiple STR profiles. Furthermore, this new version of *Familias*, can also evaluate the dropout probability [1]. Therefore this two features consolidate a great tool for the research of familiar relationships of ancient samples or samples with forensic interest with low template DNA.

An evaluation of the *Blind Search tool* of Familias 3.0 software has been performed, carrying out kinship analysis of the possible relationships between 42 individuals exhumed from 12 different burials of 6 archaeological sites from among 3000 and 4000 years of antiquity located in *Castilla y León* (Spain) [2]. The search of familiar relationships was carried out between individuals exhumed in the same tomb or between individuals buried in nearby tombs. To evaluate the results, it was taken into account also the mitochondrial DNA haplotype of each individual, the number of markers considered for the establishment of the kinship as well as the different archaeological characteristics.

1. Kling, D., Tillmar, A.O., Egelend, T. 2014. Familias 3 - Extensions and new functionality. Forensic Science International: Genetics. Vol. 13, Novembre 2014, Pages 121 -127.
2. Palomo-Díez, S., Esparza-Arroyo, A., López-Parra, A., Baeza-Richer, C Y Arroyo Pardo, E. Diversity of collective burials of the Copper and Bronce Ages on the north plateau of the Iberian Peninsula. 2013. Annali dell-

Universitá di Ferrara Museologia Scientifica e Naturalistica. ISSN 1824-2707. Vol. 9/1

Theme 13: Biostatistics

328. DEVELOPMENT OF NEW PEAK-HEIGHT MODELS FOR A CONTINUOUS METHOD OF MIXTURE INTERPRETATION

Manabe S.[1], Hamano Y.[1,2], Kawai C.[1], Morimoto C.[1], Tamaki K.[1]

[1] *Department of Forensic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan*
[2] *Forensic Science Laboratory, Kyoto Prefectural Police H.Q., Kyoto, Japan*

manabe@fp.med.kyoto-u.ac.jp

In forensics, traditional DNA mixture interpretation is based on a binary method, which does not account for peak-height information in DNA profiles. In recent years, scientifically developed countries have adopted a continuous method utilizing peak heights and considering stochastic effects (e.g., allele drop-out) to rigorously calculate likelihood ratio (LR) values. We are currently developing an open-source software program based on a continuous method and plan to share its computational principle with other researchers in the field. Here, we estimate probabilistic distributions of 3 main factors (locus specific amplification efficiency, heterozygote balance, and stutter ratio) to calculate expected allelic and stutter peak heights in the continuous method.

Single-source DNA extracted from buccal cells (n = 234) was amplified using the Identifiler Plus Kit. PCR products were then analyzed using a 3130xl Genetic Analyzer with a detection threshold of 30 relative fluorescence units (RFU). We calculated the values of the 3 main factors listed above and estimated their probabilistic distributions, based on peak heights.

Our data suggested that locus specific amplification efficiency follows a normal distribution while heterozygote balance follows a lognormal distribution for each locus. We modeled lognormal distributions for stutter ratios with allele-specific mean values, which demonstrated a positive correlation with repeat

numbers. However, with the D8S1179, D21S11, TH01, and D2S1338 loci, the simple lognormal models did not fit our data because of the complex repeat structures involved. We propose alternative models for these 4 loci and illustrate the performance of our program incorporated models for each factor.

329. BEYOND THE LIMITS OF DNA TYPING

Benschop C.[1], Haned H.[1], Sijen T.[1]

[1] Netherlands Forensic Institute, The Hague, The Netherlands

c.benschop@nfi.minvenj.nl

The interpretation of forensic DNA samples can be complex when low-template (LT) amounts of DNA and/or many contributors are present. The use of enhanced interrogation techniques for LT DNA samples improve the detection of alleles, but do not eliminate stochastic effects. To increase the amount of DNA in the amplification LT DNA samples can be concentrated by, for example, ethanol precipitation though this requires additional handling. Furthermore, with many contributors (≥three), mixture interpretation is complicated due to allele sharing.

In our studies, we generated large sets of complex DNA profiles. We studied their characteristics and calculated a likelihood ratio (LR) using the LRmix model. We examined amongst others the influence of 1) standard or enhanced settings for capillary electrophoresis, 2) replicates or concentrated samples and 3) the true or an incorrect number of contributors on the LR.

From our results it appears that 1) large LRs can be obtained using amounts of DNA that may not be meaningful from a biological or criminalistic point of view, 2) replicate analyses is not always the best option for LT DNA samples and 3) conditioning on an incorrect number of contributors is generally conservative, though may result in false-exclusions.

330. LINKED MARKERS IN MIXTURES

Dørum G.[1], Tillmar A.[2,3], Egeland T.[1,4]

[1] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Aas, Norway
[2] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linkøping, Sweden
[3] Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden
[4] Department of Forensic Biology, Norwegian Institute of Public Health, Oslo, Norway

guro.dorum@nmbu.no

Standard forensic DNA work is based on the use of unlinked markers. There are however scenarios where linked markers can be a relevant alternative. Linked markers may increase the power in relationship testing, and there are even examples of cases where different family relationships cannot be distinguished if only unlinked markers are studied. X-chromosomal markers are increasingly used in complex kinship cases to supplement the standard STR markers. The markers on the X chromosome are linked within groups.

Another advantage of using linked markers is the large number of markers that become available compared to if markers are required to be unlinked. In addition, several studies have found that some of the commonly used STR markers are linked, and ignoring this linkage may have considerable impact on the likelihood ratio.

In this work we consider the use of linked markers in cases with mixture profiles, and in particular we will look at the effect of linkage disequilibrium. Several markers on the X chromosome are known to be in linkage disequilibrium. Falsely assuming linkage equilibrium may lead to false inclusion of relationships. We study the effect that linkage disequilibrium has on likelihood ratios for different mixtures.

331. THEORY AND STATISTICS OF MUTATION RATES: A MATHEMATICAL FRAMEWORK REFORMULATION FOR FORENSIC APPLICATIONS

Pinto N.[1,2,3], Alves C.[1,2], Gusmão L.[1,2,4], Amorim A.[1,2,5]

[1] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal
[2] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal
[3] Centro de Matemática da Universidade do Porto, Porto, Portugal
[4] Laboratório de Diagnóstico por DNA (LDD), Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, Brazil
[5] Faculdade de Ciências, Universidade do Porto, Porto, Portugal

npinto@ipatimup.pt

Mutation is a fundamental topic in forensic genetics. Whilst the calculation of kinship likelihoods depends on estimates of germinal mutation rates, other situations implicate mitotic mutational events. Germinal mutation rates are computed by simple proportioning the number of true parent-child genotypic configurations inconsistent with Mendelian segregation. For the STRs, current technology is based on PCR fragment size determination and germinal mutations are detected when the parent-child do not share alleles' size. For technical reasons, it is uncommon to obtain the sequence composition of STR alleles, and the identification of the allele that mutated is based on assumptions. It has been assumed that one-step are much more common than multi-step mutations. Whenever a genotypic parent-child configuration is compatible with Mendelian rules by a single-step mutation, this is assumed, despite other possible mutational events. Multi-step events are therefore evoked exclusively when a single-step cannot reconcile the observation with the true kinship, leading to an overestimation of the single step mutation rates. For any mode of transmission other than haploid/haploid it is theoretically impossible to identify both the allele at "origin" (in parents) and at "destination" (in offspring). Therefore a more sophisticated statistical framework than the simple proportioning is then required to properly evaluate the genetic data. Moreover, the possibility of the occurrence of silent alleles must be incorporated in the model. In this work we present a theoretical formalization of the problem and suggest an approach to obtain suitable data, enabling the estimation of (bi) allelic specific mutation rates, adequate to casework application.

332. ILIR, A BIOSTATISTICAL TOOL FOR ANALYSING THE OVERALL IMPACT OF GENETIC LINKAGE FOR AN ARBITRARY SET OF MARKERS AIMED AT FORENSIC IDENTITY AND RELATIONSHIP TESTING

Tillmar A.[1,2], Phillips C.[3]

[1] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden
[2] Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden
[3] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

andreas.tillmar@rmv.se

The introduction of second-generation sequencing technology has enabled the combination of a much-expanded number of DNA markers (notably STRs and SNPs in one or combined multiplexes), with the aim to increase the weight of evidence in forensic casework. However, when data from multiple loci on the same chromosome are used, genetic linkage can affect the final likelihood calculation. ILIR, is a biostatistical tool that can be used to study the overall Impact of genetic Linkage for an arbitrary set of markers aimed at forensic Identity and Relationship testing. Application of ILIR can be useful during marker selection and design of new marker panels, as well as being highly relevant for existing marker sets in order to properly evaluate effects of linkage on a case-by-case basis. ILIR, implemented via the open source platform R, includes variation and genomic position reference data for over 50 STRs and 140 SNPs, combined with the ability to include additional forensic markers of interest. Use of the software will be demonstrated, including examples from several different established marker sets, followed by a review of recommendations for the interpretation of linked genetic data.

333. FORENSIC BIOSTATISTICS WITH CLOUD COMPUTING

Götz F.[1]

[1] *Qualitype GmbH, Dresden, Germany*

f.goetz@qualitype.de

In the past years the number of forensic cases was grown constantly and with the rapid development of high-throughput technologies, forensic investigation has generated a great amount of data. New technologies like next generation sequencing (NGS) will speed up this process and deliver an unprecedented amount of information, which must be analyzed. In addition the ongoing development of new biostatistics methods for mixture analysis, complex paternity cases or disaster victim identification cannot be solved in sufficient time with standard computer hardware of a forensic laboratory.

A way to solve this problems and to fit the future needs for modern biostatistics and the growing number of usable information can be cloud computing. These IT technologies alone can deliver the necessary computer power and storage capacity. It is also necessary to develop new biostatistic algorithms and methods to use the possibilities of that technology.

We will show several examples how cloud computing combined with new bio statistical algorithms can dramatically improve the time needed for data analysis. These include also cases which cannot be solved with typical forensic laboratory software and hardware solution in a sufficient time span.

334. A SIMPLE UNCONSTRAINED SEMI-CONTINUOUS MODEL FOR CALCULATING LIKELIHOOD RATIOS FOR COMPLEX DNA MIXTURES

Russell D. E.[1], Christensen W. J.[2]

[1] *Oklahoma City Police Department, Oklahoma City, Oklahoma, United States*

[2] *Oklahoma State Bureau of Investigation, Edmond, Oklahoma, United States*

Drus13@sbcglobal.net

With advancements in analytical techniques and with the expanded use of DNA analysis in criminal investigations, DNA mixtures are being developed from samples that contain very low amounts of template DNA. Complex mixtures present significant challenges in interpretation and in calculation of appropriate statistical weights for inclusions. Methods for calculating likelihood ratios for mixtures have been developed, but factors including the complexity of equations and assumptions about possible numbers of contributors in a sample have prevented some laboratories from embracing these proposed calculation methods. This research was focused on deriving a model for calculating likelihood ratios to provide statistical weight for inclusions by comparing the probability of a suspected contributor's profile being represented in the mixture data versus the probability of any other profile being represented by the data. The model was then tested to determine the relevance of the weights provided. The resulting model is simple, easily understood, and can be quickly calculated. This model is transparent, has no cost, and can easily be validated by any laboratory with readily available software, such as Microsoft Excel. This model was evaluated with hundreds of thousands of comparisons using real and simulated DNA mixtures with two or more contributors. The testing of this model revealed the model's limits, and by working within the established limits, appropriate weights to any inclusion were able to be calculated with a greater assurance that a false inclusion did not occur.

335. ASSESSING EVIDENTIAL STRENGTH OF A Y-CHROMOSOMAL STR MATCH

Andersen M. M.[1], Eriksen P. S.[1], Morling N.[2]

[1] *Department of Mathematical Sciences, Aalborg University, Aalborg, Denmark*
[2] *Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

mikl@math.aau.dk

There is no general agreement on how to estimate the evidential weight of a match between the Y-chromosome STRs of two individuals. Mainly two questions have been raised: Firstly, how can reliable estimates of Y-STR haplotype frequencies be obtained taking into consideration that a large proportion of the Y-STR haplotypes are rare and not necessarily represented in the reference population samples (database)? Secondly, how can we compensate for the possible lack of information concerning the relevant population referred to in an alternative hypothesis (typically 'the proposition of the defence') if the relevant population is not represented to a sufficient degree in the database used for comparison? We present results comparing different methods for estimation of Y-STR haplotype frequencies and theta values. The results are based on both real and simulated data. In addition, the effect of varying numbers of Y-STR loci is investigated.

336. THE OPEN-SOURCE SOFTWARE LR*MIX* CAN BE USED TO ANALYSE SNP MIXTURES

Gill P.[1,2], Haned H.[3], Eduardoff M.[4], Santos C.[5], Phillips C.[5], Parson W.[4,6]

[1] *Norwegian Institute of Public Health, Oslo, Norway*
[2] *Department of Forensic Medicine, University of Oslo, Norway*
[3] *Department of Human Biological Traces, Netherlands Forensic Institute, The Hague, The Netherlands*
[4] *Institute of Legal Medicine, Medical University of Innsbruck, Austria*
[5] *Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain*
[6] *Forensic Science Program, The Pennsylvania State University, PA, USA*

peterd.gill@gmail.com

A series of two- and three-person mixtures of varying dilutions were prepared and analysed with Life Technologies' HID-Ion AmpliSeq™ Identity Panel v2.2 using the Ion PGM™ massively parallel sequencing system. This panel includes 136 autosomal SNPs and 33 Y-chromosome SNPs. Using the reference samples of the donors, we evaluated the strength of evidence with likelihood ratio (LR) calculations using the open-source *LRmix* program. This program was designed for multi-allelic STRs, but can be extended to bi-allelic SNPs without modification. We conditioned on each donor in turn, as the person of interest (POI) in the numerator. The LR tests showed that two-person mixtures typically gave LRs of the order of $>10^8$ whereas three-person mixtures were highly variable in results. Approximately 50% of results gave results that were exclusionary or neutral. The remainder provided LRs ranging between 10^3–10^8. We further evaluated these mixtures using diagnostic non-contributor tests. Our preliminary work shows that simple two-person mixtures can be readily analysed with *LRmix*, but the performance of three- or more person mixtures is less predictable and may fail to provide probative evidence. However, if a higher number of loci were multiplexed, the analysis of mixtures would be improved, particularly if rare allelic variants (<0.1, >0.9) were targeted in identity SNP selection.

337. ACTIVITY LEVEL IN THE HIERARCHY OF PROPOSITIONS IN CASE OF THE PEOPLE OF THE STATE OF CALIFORNIA V. ORENTHAL JAMES SIMPSON ANALYZED USING BAYESIAN NETWORK

Drábek J.[1]

[1] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, the Czech Republic*

Jiri.drabek@upol.cz

DNA profiling usually rests on source or sub-source level in the hierarchy of propositions and when used as evidence for level of activity and offence, much of its seemingly impenetrable power may be lost (1). Fully Bayesian approach of evidence analysis for higher level propositions is achievable using Bayesian network. However, forensic geneticist alone does not have sufficient data and expertise to perform such analysis in real case scenario. Moreover, they would

overstep the role of scientist in the judicial process.

With a benefit of hindsight, it is possible to perform such kind of analysis for highly publicised cases. Such case is one of the former professional football star and actor O. J. Simpson who was tried on two counts of murder (of his ex-wife, Nicole Brown Simpson, and waiter Ronald Lyle Goldman, in June 1994).

Here I present Bayesian network created on the basis of two other Bayesian nets (2,3) and information from Wikipedia. Five separate Bayesian nets (Motive; Occasion and Escape; Gloves; Knife; and Bloodstains, hairs, and fibres) are combined to yield the total likelihood ratio of over seven bans what would change 50% prior to 99.99999% posterior probability of O. J. Simpson stabbing two persons to death.

Analysis of the mistakes made by prosecution and skills of defense are outside of the topic of this contribution.

Acknowledgements: Supported by Czech grants LO1304, CZ.1.05/3.1.00/14.0307, IGA NT13569, LM2010004, and TE02000058.

1. Evett IW, Gill PD, Jackson G, Whitaker J, Champod C. Interpreting small quantities of DNA: the hierarchy of propositions and the use of Bayesian networks. J Forensic Sci 2002; 47(3):520-530.
2. Thagard P. Why wasn´t O.J. convicted? Emotional coherence and legal inference. Cognition and Emotion 2003; 17:361-383.
3. Olumide K. A probabilistic and graphical analysis of evidence in O. J. Simpson´s murder case using Bayesian networks. The Florida State University. College of Arts and Sciences, 2010.

338. PARAMETRIC APPROACH TO KINSHIP TESTING

Garcia-Magariños M.[1,2], Egeland T.[1], López-de-Ullibarri I.[2], Salas A.[3]

[1] IKBM, Norwegian University of Life Sciences, ºAs, Norway
[2] Departamento de Matemáticas, Universidade da Coruña, Spain
[3] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain

manugm1981@hotmail.com, antonio.salas@usc.es

Statistical inference of family relationships has traditionally followed a likelihood-based formula, dating back to Essen-Moller. This classical approach relies on a verbal formulation of the problem, instead of being parameter-based. Nevertheless, the classical theory of statistics usually states the null and alternative hypothesis by means of parameters and so there is a large theory that can be applied directly. A new hypothesis testing approach based on identity-by-descent (IBD) parameters has been developed here. The use of IBD probabilities to specify family relationships was first developed by Cotterman and has subsequently been studied by Thompson. Mathematical properties of the test have been thoroughly studied in order to provide with theoretical consistency. Study of the test statistic distribution under the null hypothesis is essential to obtain a p-value used to take an informed decision. Performance of the testing approach on different simulated scenarios and a real dataset consisting of gypsy children has been studied. Comparisons with the classical approach have been established. Results indicate this new testing approach is highly competitive.

339. COMPARISON OF TWO ONLINE ALGORITHM METHODS FOR FORENSIC ANCESTRY INFERENCE

Yun L.[1,2], Gao T.[2], Sun K.[2], Gu Y.[2], Hou Y.[2]

[1] Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science of Ministry of Justice, Shanghai, China
[2] Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

profhou@yahoo.com, forensic@scu.edu.cn

The forensic prediction of the biogeographic ancestry based on DNA typing has become more widespread. The search for optimized panels, consisting of a small but efficient and robust set of ancestry informative markers (AIMs), has

received increased interest. Several panels published in recent literature would provide excellent information on ancestry as a useful forensic investigative tool. Relying on the genetic profiles from these panels, accurate and efficient estimation of an individual ancestry depends on excellent algorithmic methods. In the present work, a comparison of the FROG-kb (http://frog.med.yale.edu) and Bayesian classification approaches of Snipper (http://mathgene.usc.es/snipper/) was carried out to assess the ability of genetic ancestry inference for the Kidd Lab 55 AISNP panel (Kidd et al., FSI Genetics 2014 (10), 23-32). Preliminary reported data from the online SPSmart browser illustrated that both algorithmic inference methods were adaptable for forensic ancestry assignments of an individual. For a few individuals, especially originating from admixed populations, ancestral assignment was inaccurate or discordant according to the likelihood calculations in FROG-kb or the ratio from Snipper forensic ancestry analysis portal. Therefore, future improvement will require more populations adding into the reference populations for the likelihood function in FROG-kb, as well as appropriate training sets applying to the online Snipper analysis tool.

340. INVESTIGATION OF THE MIXTURE INTERPRETATION COMPATIBILITY WITH VALIDATION AND SIMULATION STUDIES

Zorlu T., Bulbul O., Rayimoglu G., Filoglu G.

Istanbul University, Institute of Forensic Sciences, İstanbul, Turkey

tolgazorlu1@gmail.com

Interpretation of the DNA evidence is the most important part of the analytical process in forensic sciences. DNA analysts can generally encounter with mixed DNA samples. Related samples can include 2 or more individuals DNA profiles. On last decade, most of the European countries and also in U.S.A court systems benefit from various statistical approaches on forensic DNA analysis. Since these approaches can't be performed without any validation process, the compatibility of validation studies play big role on interpretation period. In this study, simulated mixed samples were interpreted and compared with mixture interpretation validation studies in order to maintain the feasibility of interpretation process. A brief validation process was performed in order to maintain DNA typing kit and laboratory parameters and thresholds. After the validation process mixed DNA samples were formed on different ratios. DNA samples were analyzed with AmpFℓSTR® Identifiler kit and analyzed with Genemapper® IDX v.1 software on ABI 3130 genetic analyzer. The statistical interpretation of mixtures was performed with LRmix™ module. The most frequent allelic disorientation was determined as allele drop-outs on the different mixture ratios. Comparison of the simulated and validation samples were shown different results on both on detected alleles and LR value. Finally likelihood ratio and LRmix module was determined as useful and successful application on both mixture samples. Furthermore more specific validation procedures are required in order to increase both consistency and accuracy of the mixture interpretation on laboratories.

341. IS PEAK HEIGHT IMPORTANT FOR THE STATISTICAL EVALUATION OF THE WEIGHT OF EVIDENCE IN DNA MIXTURES?

Caglià A.[1,2], Baldassarri L.[1], Boschi I.[1], Scarnicci F.[1], Pascali V. L.[1]

[1] *Institute of Legal Medicine, Catholic University of Sacred Heart, Rome, Italy*
[2] *Forensic Science Police Service, Ministry of the Interior, Rome, Italy*

alessandra.caglia@interno.it

In order to evaluate how and to what extent the alleles peak height can be a useful information to calculate the LR value, some real and high-quality "balanced" and "unbalanced" mixtures containing two contributors were subjected to statistical analysis.

To calculate the LR value for the real suspect(S1), under the basic hypothesis HpS1U/HdUU, we used first the free on-line analysis software LRmix study, while, for the quantitative approach, a ready-made Microsoft excel spreadsheet

accounting for the peaks heights of profiles.

At a second stage, we replaced the true suspect(S1) with a mock-suspect(S2), specifically designed to be genetically compatible but highly unlikely as a genotype from a quantitative point of view. The LR value was recalculated under the same circumstances and with the same analysis parameters.

When comparing true and fake results, the LR value obtained with a uniform approach (without peaks heights) had substantially the same order of magnitude in both mixtures using S1 and S2, as well as in the "balanced" mixtures analyzed using the quantitative approach, due essentially to the random allele frequencies combinations.

On the contrary, the LR undergoes a slight decrease using S1 and a substantial decrease using S2 (as well as in the reality of the facts) in the "unbalanced" mixtures analyzed using the quantitative approach.

This implies that the quantitative information expressed by the peaks heights does not seem to be particularly useful in the case of the "balanced" mixed profiles, but seems to have crucial importance in the case of the "unbalanced" mixtures.

342. COMPLEX DNA MIXTURE ANALYSIS: REPORT OF TWO CASES

Tasselli G.[1], Tommolini F.[1], Margiotta G.[2], Lancia M.[1], Massetti S.[1], Carnevali E.[1]

[1] *Section of Legal Medicine, University of Perugia, Italy*
[2] *Section of Legal Medicine, University of Siena, Italy*

dr.massimolancia@gmail.com

In the last years, as DNA profiling techniques become more sensitive, mixtures by multiple contributors become the norm (1). Nevertheless, interpretation of mixtures profiles has proven to be the most complex tasks in the forensic field (2). The ISFG have published recommendations for the interpretation of mixed DNA profiles that may have allelic drop-out or drop-in. The DNA commission of the ISFG has supported the use of likelihood ratios (LRs), which were deemed preferable to other methods (3). The LR is considered to be the most powerful and relevant measure of the weight of evidence. Likelihood ratios can be calculated by probabilistic programs, using semi and fully continuous (probabilistic) methods. These methods increase the efficiency of forensic laboratories, and improve the consistency and transparency of the reported results. LRmix is an open-source system dedicated to the interpretation of forensic DNA profiles, with a particular focus on complex DNA mixtures. On 2014, 15th December was allowed the first release of LRmix Studio (4). This software , likely LRmix, is programmed on the likelihood ratio model described by (3) and (1), and facilitates the calculation of likelihood ratios for complex mixtures, with partial profiles with known and unknown contributors. Here, we report the re-analysis of two old complex cases processed in our laboratory in the last years. In both cases, we used the open-source software LRmix Studio with interesting consequences: in fact, in one case we solved the crime, and in the other one, the results allowed the case to be reopened.

1. Gill P, Haned H. A new methodological framework to interpret complex DNA profiles using likelihood ratios. Forensic Sci Int Genet. 2013 Feb;7(2):251-63.
2. Prieto L, Haned H, Mosquera A, Crespillo M, Alemañ M, Aler M, Alvarez F, Baeza-Richer C, Dominguez A, Doutremepuich C, Farfán MJ, Fenger-Grøn M, García-Ganivet JM, González-Moya E, Hombreiro L, Lareu MV, Martínez-Jarreta B, Merigioli S, Milans Del Bosch P, Morling N, Muñoz-Nieto M, Ortega-González E, Pedrosa S, Pérez R, Solís C, Yurrebaso I, Gill P. Euroforgen-NoE collaborative exercise on LRmix to demonstrate standardization of the interpretation of complex DNA profiles. Forensic Sci Int Genet. 2014 Mar;9:47-54.
3. Haned H, Benschop CC, Gill PD, Sijen T. Complex DNA mixture analysis in a forensic context: Evaluating the probative value using a likelihood ratio model. Forensic Sci Int Genet. 2014 Nov 24;16C:17-25.
4. http://lrmixstudio.org/download/

343: ACCOUNTING FOR LINKAGE BETWEEN THE STR LOCI D5S818/CSF1PO AND VWA/ D12S391 IN KINSHIP ANALYSES: IMPACT ON LIKELIHOOD RATIO VALUES

Burri H.[1], Sulzer A.[1], Voegeli P.[1], Kratzer A.[1]

[1] *Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland*

helen.burri@irm.uzh.ch

For kinship testing, it is important to consider the impact of genetic linkage when evaluating data from closely positioned STR loci. Various commercially available multiplex kits contain pairs of loci on the same chromosome, which has raised concerns about whether or not such markers can be treated as independent loci in kinship analyses. The aim of our study was to evaluate the impact of ignoring versus accounting for linkage on likelihood ratio (LR) values. We took linkage into account by incorporating the recombination frequency (*R*) into LR calculations. For 65 pairs of full siblings and 65 pairs of unrelated persons, we examined the markers D5S818 and CSF1PO and computed LR values for the pedigree scenario "full siblings" versus "unrelated". The effect of ignoring versus accounting for linkage (R = 0.252), expressed as the ratio of $LR_{unlinked}/LR_{linked}$, ranged from 0.64 to 1.31 for the pairs of full siblings (mean = 0.97) and from 0.64 to 1.54 for the pairs of unrelated persons (mean = 0.91). In addition, we calculated LR values for the loci vWA and D12S391 in 46 cases of kinship testing encountered at our institute where linkage considerations were expected to affect the LR. On average, LR values ignoring linkage were slightly underestimated compared to LR values accounting for linkage (R = 0.117). LR ratios ($LR_{unlinked}/_{LRlinked}$) ranged from 0.47 to 1.96 (mean = 0.84). Furthermore, we evaluated the effect on LR values if only one of two closely positioned loci was included in a kinship analysis.

344. DROP-OUT MODEL OPTIMIZATION ON DEGRADED DNA SAMPLES

Duchamp V.[1,2], Bastisch I.[1], Schneider P. M.[2]

[1] *DNA Unit, Federal Criminal Police Office (BKA), Wiesbaden, Germany*
[2] *Institute of Legal Medicine, University of Cologne, Cologne, Germany*

peter.schneider@uk-koeln.de

Samples collected during a crime scene investigation present different challenges both during the DNA analysis process and the result interpretation. The resulting DNA profiles can be incomplete due to a low amount of DNA or present signs of DNA degradation. It can also indicate a DNA mixture of two or more people.

Due to a low amount of DNA and/or associated with degradation, a common phenomenon called drop-out appears. It can concern one or several alleles or loci. Drop-out is a major problem particularly when differences are observed comparing a trace profile with a reference profile. These differences need to be evaluated and explained.

This study is based on experimental data trying to reproduce samples possibly found on a crime scene. Four saliva swabs were taken from ten voluntary donors and exposed during 3-6-9-12 weeks to humidity at 37°C. Then the 40 samples were extracted, amplified and analysed using the NGM-Select multiplex kit using standard procedures.

The aim of this research is to present a logistic regression model providing a probability of drop-out for degraded DNA samples. This model will be based on explanatory variable such as the peak height, the DNA quantity, the allele length and dyes. The logistic regression is a tool commonly used to model binary data, like drop-out; an allele or a locus can be either present or absent. The model is elaborated using the open-source software R, widely used in the forensic community.

345. OPEN SOFTWARE FOR RELATIONSHIP INFERENCE BASED ON MIXTURES

Kaur N.[1], Bouzga M.[2], Dørum G.[1], Egeland T.[1,2]

[1] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Aas, Norway
[2] Department of Forensic Biology, Norwegian Institute of Public Health, Oslo, Norway

navreet.kaur@nmbu.no

Kinship cases involving DNA-mixtures are in general rarely seen. However, in a case handled by the Norwegian Institute of Public Health, sample DNA was obtained after a rape case that resulted in a pregnancy and abortion. A paternity test was performed to resolve the case. The problem, however, was that the DNA from the fetus came in form of a mixture with the mother, and the mother (being the victim) refused to give her reference data. As this case illustrates, kinship cases involving mixtures and missing reference profiles do occur and makes the use of existing methods rather inconvenient. The introductory rape case was solved by developing new statistical methods that may handle general relationship inference based on DNA-mixtures. We here emphasize the implementation of the statistical methods developed. The basic idea is that likelihood calculations can be decomposed into a series of kinship problems. To do so, we make use of already existing kinship open software (the R-version of Familias) in our R library relMix. We are then able to handle complicating factors like mutations, silent alleles and kinship correction more easily. We exemplify the methods and their implementation using the data gained from the rape case, and show the reader how the methods can be used for different kinds of relationship inference.

1. Mostad, P. and Egeland, T.: Familias: Probabilities for Pedigrees Given DNA Data.R package version 2.2. http://CRAN.R-project.org/package=Familias. 2015
2. Hu, Y. Q., Fung, W. K., and Choy, Y. T.: Interpreting DNA mixtures with relatives of a missing suspect. Remote Sensing, Environment and Transportation Engineering (RSETE). 2011: International Conference on: pages 7649-7652.
3. Egeland, T., Dørum, G., Vigeland, M.D., and Sheehan, N.A.: Mixtures with Relatives: A pedigree perspective. Forensic Science International: Genetics. 2014; 10: pages 49-54.

346. THE DARK SIDE OF DNA INTERPRETATION IN COMPLEX COLD CASES: "THE RISK OF A WRONG IDENTIFICATION WHEN FACING WITH LT-DNA MIXTURES"

Garofano P.[1,4], Testi R.[1,2,4], Risso E.[1,3], Vincenti M.[4,5], Alladio E.[4,5], Garofano L.[1]

[1] Accademia Italiana di Scienze Forensi, Reggio Emilia, Italy
[2] S.C. Medicina Legale, ASL TO2, Torino, Italy
[3] Department of Forensic Sciences, University of Split, Split, Republic of Croatia
[4] Laboratorio Biologia Forense - Centro Regionale Antidoping "A. Bertinaria", Orbassano (TO), Italy
[5] Dipartimento di Chimica, Università degli Studi di Torino, Torino, Italy

ealladio@unito.it

DNA typing interpretation plays a fundamental role, particularly in cold cases incorrectly analyzed during crime scene investigation activities and, moreover, wrongly processed during laboratory analyses. LT-DNA mixture profiles collected at the crime scene represent the most challenging situation for the DNA analyst[1-3], since, according to different interpretation procedures, the outcome of the Trial can change dramatically. The interpretation may be extremely difficult when facing cold cases, especially if several variables are present, such as random sample degradation and/or contamination due to inexperienced investigators and multiple forensic re-examinations following previous mistakes made processing the evidence.

This work describe the difficult approach to a famous Italian murder case, connected to the Mafia organization, which took place in Sicily 25 years ago.

DNA analyses were performed on the barrels of a rifle collected at the crime scene, which was largely manipulated during the sampling as well as in the laboratory, when fingerprint and ballistic analyses were performed, resulting in widespread DNA contamination of the evidence. Three main models[4-6] of DNA mixtures data interpretation – binary, semi-continuous, fully continuous – were adopted giving unbiased interpretations. Our study highlight that results can widely change when different statistical models and parameters are involved particularly in complex cases; this methodology allowed to evaluate the effective statistical inference accidentally caused by external contributors due to the wide accidental contamination. Deep validation studies both on mixtures and degraded samples gave us more details for a multi-software interpretation approach for challenging samples.

1. Budowle B, Onorato AJ, Callaghan TF, Della Manna A, Gross AM, Guerrieri RA, Luttman JC and McClure DL: Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework. J. Forensic Sci. 2009; 54: 810 – 821.
2. Balding D: Evaluation of mixed-source, low-template DNA profiles in forensic science. J. Natl. Acad. Sci. 2013; 110: 12241 – 12246.
3. Gill P, Puch-Solis R and Curran J: The low-template-DNA (stochastic) threshold–Its determination relative to risk analysis for national DNA databases. Forensic Sci. Int. Genet. 2009; 3: 104 – 111.
4. Gill P and Haned H: A new methodological framework to interpret complex DNA profiles using likelihood ratios. Forensic Sci. Int. Genet. 2013; 7: 251–263.
5. Kelly H, Bright J, Buckleton J and Curran J: A comparison of statistical models for the analysis of complex forensic DNA profiles. Sci. Justice. 2014; 54: 66 – 70.
6. Taylor D: Using continuous DNA interpretation methods to revisit likelihood ratio behavior. Forensic Sci. Int. Genet. 2014; 11: 144 – 153.

347. AN ALTERNATIVE APPLICATION OF THE CONSENSUS METHOD TO DNA TYPING INTERPRETATION FOR LT-DNA MIXTURES

Garofano P.[1,2], Caneparo D.[1,3], D'Amico G.[1], Vincenti M.[1,3], Alladio E.[1,3]

[1] Laboratorio Biologia Forense - Centro Regionale Antidoping "A. Bertinaria", Orbassano (TO), Italy
[2] Accademia Italiana di Scienze Forensi, Reggio Emilia, Italy
[3] Dipartimento di Chimica, Università degli Studi di Torino, Torino, Italy

ealladio@unito.it

The interpretation approach to evaluate LT-DNA mixtures is one of the most largely debated and investigated topics in forensic genetics[1-4]. Evaluating profiles obtained from LT-DNA mixtures several parameters where stochastic phenomena are involved, such as drop-in and drop-out, must necessarily be taken into account according to thresholds adopted from the lab. In order to adopt a rigorous approach to LT-DNA mixture interpretation and after deep validation studies, in our laboratory we opted for a parallel use of two different models reported in literature, such as the semi-continuous approach[5,6] (using LRmixStudio© and LabRetriever© software) and the fully-continuous approach[7,8] (DNA•VIEW™ "Mixture solution" software). These models present different difficulty degrees in terms of application and interpretation. Parallel software use and multi method approach helped us to highlight valuable interpretation key in evaluating profiles obtained. Moreover, we clearly understood the extreme caution needed in order to achieve a correct interpretation of DNA profiling results in complex and challenging samples particularly why they may heavily affect the outcome of a Trial. According to our validation studies and literature, we developed a so-called "*consensus model*" for DNA interpretation.

In particular, our consensus approach consists on LR values comparison provided by different software and, only when all the LR results obtained turn out to be similar and convergent, we take into account the most conservative one. On the contrary, if the LR results are not convergent, the DNA interpretation is inconclusive. We successfully applied this method both on validation studies

and on real cases.

1. Butler JM: Advanced Topics in Forensic DNA Typing: Interpretation. London, UK: Elsevier Academic Press; 2014: 1 – 608.
2. Triggs C.M., Buckleton J.S., Walsh S.J: Forensic DNA Evidence Interpretation.CRC Press; 2005: 1 – 552.
3. Pfeifer CM, Klein-Unseld R, Klintschar M and Wiegand P: Comparison of different interpretation strategies for low template DNA mixtures. Forensic Sci. Int. Genet. 2012; 6: 716 – 22.
4. Curran JM, Gill P and Bill MR: Interpretation of repeat measurement DNA evidence allowing for multiple contributors and population substructure. Forensic Sci. Int. 2005; 10: 47 – 53.
5. Gill P, Curran J, Neumann C, Kirkham A, Clayton T, Whitaker J and Lambert J: Interpretation of complex DNA profiles using empirical models and a method to measure their robustness. Forensic Sci. Int. Genet. 2008; 2: 91 – 103.
6. Haned H and Gill P: Analysis of complex DNA mixtures using the Forensim package. Forensic Sci. Int. Genet. Supplement Series. 2011; 3: e79 - e80.
7. Brenner CH: Forensic Genetics: Mathematics Brenner, Charles. In: Encyclopedia of Life Sciences. Chichester, UK: John Wiley & Sons Ltd, 2006.
8. Brenner CH: DAVIEW™ DNA Identification Analysis Programs, 2011.

348. THE DNA·VIEW MIXTURE SOLUTION

Brenner C.[1,2]

[1] *DNA·VIEW, Oakland, California, USA*
[2] *UC Berkeley Human Rights Center, Berkeley, California, USA*

c@dna-view.com

The DNA·VIEW Mixture Solution is a computer program for solving mixture problems by a "continuous" approach – that is, the calculation takes into account the significance of peak height including varying contribution amounts along with random peak height variation. The modeling approach for likelihood calculations, based mainly on stochastic variation, incorporates dropout, drop-in, stutter and allelic stacking naturally and without additional "moving parts." Benefits of the simple and coherent design include robustness (not very sensitive to parameter values) and very fast execution speed. The program does not employ MCMC with its attendant uncertainty and opaqueness. It has several unique features including case-specific visual aids such as graphical depictions, readily understandable by any analyst, showing how various hypotheses fit or don't fit the mixture data.

349. EFFECTS OF GENETIC LINKAGE IN SIB TEST USING THE GLOBALFILER KIT

Tamura T.[1,2], Osawa M.[1], Ochiai E.[1], Miyashita K.[1], Suzuki T.[2], Nakamura T.[2]

[1] *Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Japan*
[2] *Scientific Crime Laboratory, Kanagawa Prefectural Police, Yokohama, Japan*

tamura-t@tokai-u.jp

GlobalFiler (Life Technologies) is composed of 21 autosomal STR loci where six loci have been added to the 15 loci of Identifiler. In contrast to one pair of linked loci of CSF1PO–D5S818 in Identifiler, GlobalFiler contains two other potential pairs of D2S441–TPOX and D12S391–vWA. In this study, the combined impact of the three linkages in sib test was evaluated upon simulated STR genotype data.

For a pair of linked loci, the recombination rate should be taken into account for the likelihood ratio (LR) calculation, tentatively designated as LR "exact". When the linkage was ignored, LRs can be simply multiplied according to the product rule, tentatively designated as LR "rough". The effect of linkage was evaluated by the ratio of LR "rough"/LR "exact". Then, the combined impact was estimated by calculating the values on the data for all linked loci.

In the simulated sib pairs, the median of the ratio of LR "rough"/LR "exact" were

0.87, 0.96, and 1.00 for D12S391–vWA, CSF1PO–D5S818, and D2S441–TPOX, respectively. As extraordinary values, maximum ones were 4.98, 1.63, and 1.01 for D12S391–vWA, CSF1PO–D5S818, and D2S441–TPOX, respectively. If the linkages were ignored, the LR value was possibly overestimated up to 5.83 times the LR considering the combined impact of the linkages.

The present simulation analysis demonstrated that the combined impact might be greater than expected in the previous one pair of linked loci. More careful inference should be necessary for the application of additional linked loci.

Theme 14: Paternity

350. MUTATION RATES AT 17 STR LOCI IN THE BELARUSSIAN POPULATION

Veremeichyk V. M., Borovko S. R., Kuzub N. N.

State Committee of Forensic Examinations of the Republic of Belarus, Minsk, Belarus

s_borovko@mail.ru

Autosomal loci with short tandem repeats (STR) are widely used for the human identification. Knowledge about the locus-specific mutation rates of STRs improves forensic probability calculations for the correct interpretation of diversity data. Mutations of 17 STRs contained in two conventional kits of the AmpFlSTR®Identifiler® and PowerPlex®16 systems were studied in 11,488 paternity investigation cases carried out during 2005-2014 in Belarus. In total, 276 mutations were identified at 15 of the 17 loci. Among them, 199 mutations were from paternal source, 45 from maternal source, and the rest (32) were undeterminable. The highest mutation rate was observed at FGA, and the lowest – at PentaE. The average mutation rate for each of the loci was 0.09%. No mutation was observed at the TH01 and TPOX loci. The majority (98.19%) of mutated alleles were the results of one-step mutation with the ratio of gain versus loss 1.079:1. The multiple-step mutations were found three times (2, 3 and 4 repeats). Not stepwise mutations were found twice. Double mutations were observed four times. Two different mutation events were found twice: one case in two children (FGA and D21S11) and one case in both parents (D16S539 and D18S51). 3 simultaneous mutations in the child were identified one time with two maternal mutations and one - paternal. The mutation event is very crucial for the forensic DNA analysis and accumulation of STR mutation data is important for the definition of exclusion criteria in paternity testing and for genetic profile interpretation.

351. MUTATION OF TETRA-NUCLEOTIDE STR IN PARENT-CHILD RELATION USING GLOBALFILER KIT

Ochiai E.[1], Tamura T.[1,2], Umetsu K.[3], Matsushima Y.[1], Satoh F.[1], Osawa M.[1]

[1] Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan
[2] Forensic Biology Unit, Scientific Crime Laboratory, Kanagawa Prefectural Police, Yokohama, Kanagawa, Japan
[3] Department of Forensic Medicine, Yamagata University School of Medicine, Yamagata, Japan

ochiai_e@tokai-u.jp

Tetra-nucleotide short tandem repeat (STR) polymorphisms have served as the universal DNA marker in forensic genetics. Advanced analysis kits that contain several additional loci have been available, but mutational events in parent-child relation is presumably more frequent. In the present study, occurrence of repeat number mutation is examined using GlobalFiler PCR Amplification Kit (Life Technologies). The GlobalFiler and AmpFℓSTR Identifiler PCR Amplification Kit were employed for the personal identification at postmortem. This project has been approved by the ethical committee of Tokai University School of Medicine. STR profile was determined in a total of 177 cases of Japanese parent-child relation, which were comprised of 58 father-child cases, 95 mother-child cases and 24 trio cases. Eight events of repeat number exchange were detected, which might be originated from the mechanism of slippage in replication of the germline. D12S391 (21 to 20) and SE33 (18 or 20 to 19) were included in GlobalFiler Kit only. Of the eight cases, six were paternal origin and the other two were maternal. The incidences counted to be 7.32×10^{-2} and 1.68×10^{-2} for paternity and maternity, respectively. This value was higher than Identifiler ones (4.88×10^{-2} for paternal). The GlobalFiler kit increase the discrimination power, but an inference should be more careful because of the more frequent mutational events.

352. A GENETIC INCONSISTENCY AT THE D7S820 LOCUS – MULTI-STEP MUTATION, UNIPARENTAL ISODISOMY OR OTHER?

Dauber E. M., Glock B., Mayr W. R.

Department of Blood Group Serology and Transfusion Medicine, Medical University Vienna, Austria

eva-maria.dauber@meduniwien.ac.at

In relationship testing based on STR typing isolated genetic inconsistencies between two generations might be observed. In most cases they can be explained by de novo mutations in the germline, mostly single or double step mutations. Multistep mutations are very rare events.

In a classical trio case all of 18 STR loci investigated were in concordance with the Mendelian way of inheritance except an isolated genetic inconsistency at the D7S820 locus. Both parents were heterozygous (mother *8,*11, father *12,*13) but the child was apparently homozygous (*8,*8) for an allele which it only shared with the mother. Only a four (or five) step mutation could explain the inheritance of a paternal allele in case of true paternity. This observation was very unusual in many ways: (1) this STR locus has a very low mutation rate, (2) four step mutations are extremely rare, (3) the child was apparently homozygous for a maternal allele. Given true paternity such a constellation could also be explained by uniparental isodisomy. In such cases both chromosomes, or parts of them are derived from the same parent. But also a new mutation (point mutation, deletion or insertion) in the paternal germline mutation which affects the primer binding site could result in a loss of amplification of the paternal allele.

Additonal marker systems were tested to corroborate true paternity. All D7S820 alleles involved in this case were sequenced in order to trace back the parental origin of the inherited alleles and possible mutations in the primer binding site. Furthermore other chromosome 7 markers were tested to address the possibility of uniparental isodisomy.

353. THE APPLICATION OF MULTI-INDEL AS SUPPLEMENTARY IN PATERNITY CASES WITH STR MUTATION

Gao T., Yun L., He W., Gu Y., Hou Y.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

profhou@yahoo.com, forensic@scu.edu.cn

Insertion-deletion (InDel) markers, which combine advantages of both short tandem repeats (STR) and single nucleotide polymorphisms (SNP), are suitable for personal identification and parentage testing. To overcome the limitation of poor biostatistical power for diallelic InDel, higher polymorphic multi-InDel markers with more than 3 allele markers have been successfully designed and developed. In this study, suspect one-step mutations at the Penta E locus (18 to 19, 15 to 16) were observed in two paternity cases detected with the AGCU Expressmarker kit (AGCU, China) including 22 STRs. A multiplex consisting of 20 multi-InDel loci was applied to two paternity cases. Full profiles of 20 multi-InDel markers were obtained and no mutations were found. The paternity index (PI) increased from 618 to 71226 and from 2475 to 335775 by combining both STR and multi-Indel markers, respectively. So the multi-Indels provided efficient genetic information for forensic paternity study. The results underline the importance of the multi-InDel multiplex as a supplementary to STR in special cases.

354. SIBLING INCEST

Cosentino E., Turrina S.[1]., Ferrian M., Caratti S., De Leo D.

[1] Department of Public Health and Community Medicine, Institute of Legal Medicine, Forensic Genetics Laboratory, University of Verona

stefania.turrina@univr.it

"Incest" means the sexual union between family members or close relatives, as well as among people involved in a consanguineous relationship. Sibling incest is rarely reported and usually involve abuse or coercion of young female children by older brothers. Hence is described a sibling incest case between two minors originating from Ivory Coast. A 12 years old girl gave birth to a female newborn without providing information about the identity of the putative father. A police investigation was opened and suspect fell on male subjects of her own family. Therefore the judge demanded a paternity testing to establish which between the father or the brother of the girl might have abused her. After the father's victim refused to provide his own DNA sample, genetic testing was conducted on the victim, her daughter and her brother by the analysis of autosomal STRs (PowerPlex Fusion System, Promega). Identical sharing at all the 22 loci of paternally inherited alleles between the newborn and the brother was evident. However, no certainty about his role was possible considering the close familial relationship and the absence of genetic data from the girl's father. The supplementary analysis of X-STRs (Investigator Argus X-12 kit, Qiagen) proved to be determinant in confirming the brother as the rapist of the minor girl and the newborn's father. At the end of all genetic forensic analysis the two minor's parents affirmed the constant desire of their son to "fall asleep" with his sister.

355. TWO MOTHERS AND TWO FATHERS FOR TWO HALF-BROTHERS' PAIRS

Ferrian M., Turrina S.[1], Caratti S., Cosentino E., De Leo D.

[1] Department of Public Health and Community Medicine, Institute of Legal Medicine, Forensic Genetics Laboratory, University of Verona

stefania.turrina@univr.it

Here is presented a deficient paternity case requested by a son born out of wedlock. In order to avoid exhumation of the alleged father, the judge demanded to obtain his biological profile from the analysis of his closest living relatives. Paternity testing involved the applicant (Fm), his mother (M) and two sons born in wedlock of the deceased father (Fp1, Fp2). The

analysis of 23 Y-STRs by means of the PowerPlex Y23 System revealed a full sharing of Y-chromosomal profiles between the applicant (Fm) and one son of the deceased father (Fp1), but no compatibility with the second (Fp2), thus suggesting the presence of an additional alleged father.

Giving the impossibility to establish which between Fp1 and Fp2 was the true biological son and, consequently, which 23 Y-STR profile matched with the deceased father, the judge disposed exhumation of the body.

Full identity of Y-STRs was revealed among the deceased, Fp1 and Fm, whilst Fp2 could be excluded from the lineage.

A following calculation of Likelihood Ratios achieved from the analysis with 22 autosomal STRs (PowerPlex Fusion System) allowed complete solving of the paternity case. Fm-Fp1 were found to be half-brothers with the same father, Fp1-Fp2 half-brothers sharing a common mother and Fm-Fp2 were declared unrelated.

358. SCREENING AGE-RELATED CPGS FOR FORENSIC PURPOSE IN CHINESE HAN POPULATION

Huang Y., Yan J., Zhu H., Fu X., Hou Y.

Institute of Forensic Medicine, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

profhou@ yahoo.com, forensic@scu.edu.cn

Age-related CpGs (AR-CpGs) have been proved to be potential markers for forensic age prediction. However, DNA methylation (DNAm) patterns in particular AR-CpGs may be tissue specific. Meanwhile, variant methylation quantitative platforms might result in discrepant candidate markers. Recent researches carried out either by pyrosequencing or by microarray platforms were based on Caucasian samples. In this study, we aimed to screen out the AR-CpGs as potential forensic age prediction biomarkers in Chinese Han population by pyrosequencing approach. We referred the previous age-related DNAm studies with different methods or tissue specimens, candidate markers located on 6 gene fragments were selected for DNA methylation analysis. The blood samples both from younger donors aged from 10 to 25 years and from senior donors aged from 55 to 65 years were employed to screen the candidate markers with remarkable difference in methylation values between the two age groups. Our results revealed that there were 7 CpG sites resided on 3 gene fragments showed significant difference between the adolescent group and the elderly group. It implied that some of them may be potential markers for forensic age prediction in Chinese Han population and that pyrosequencing was an effective method to screen AR-CpGs and quantify the methylation values of them.

359. HETEROPATERNAL SUPERFECUNDATION: IMPLICANCIES IN FORENSIC GENETICS

García M. G.[1], Bolontrade A. J.[2], Penacino G.[3], Chiesa I. J.[1], Pérez M. S.[1]

[1] Departamento de Medicina Genómica, laboratorio MANLAB, CABA, Buenos Aires, Argentina
[2] Laboratorio Bolontrade, General Belgrano, Argentina
[3] Colegio Oficial de Farmacéuticos y Bioquímicos / Fundacion INGEN, Buenos Aires, Argentina. E-mail presidencia@ingenfoundation.org

Corresponding author: María Gabriela García. Departamento de Medicina Genómica, laboratorio MANLAB. Marcelo T. de Alvear 2263 (1122 Buenos Aires, Argentina. Te: + 5411 4511-9661

e-mail: gabriela.garcia@manlab.com.ar.

A marked increase in multiple pregnancies has been observed in recent years with significant variations between countries worldwide, mainly due to the expansion of assisted reproduction techniques and, to a lesser extent, delayed motherhood onset. In cases of paternity research, different biological ties, and cases regarding human identification, this suggests an increase of twin brothers involved in this type of study. Occasionally, it has been seen in many court cases involving twins' participation, the attempt to prove that tested

paternity to the alleged father for one of them, necessarily implies the same result for the other. This usually occurs when one of the twins is not available for affiliation studies due to different reasons, such as death, missing, illness or other. This article describes a rare event called heteropaternal superfecundation (SFH) detected in a case of paternity investigation conducted in our laboratory.

In order to establish the link paternity between a pair of dizygotic twins, with different sex, four months old and an alleged father, our laboratory was consulted about the possibility of conducting the study with the participation of only one of the minors as it would be less difficult for the mother to bring one of the babies, and especially because in that way the cost of the study would be lower. The mother believed that the result of one of the babies, since they were twins, could be extended to the other child. After explaining the reasons why this was not possible, she agreed to do it with the participation of both.

The Alleged father-twin 1 pair showed discrepancies in 14 STR loci for Y chromosome and in 11 autosomal STR markers.

The genetic profile in twin 2 showed a 50 % match with her mothers' and a 50% with the alleged fathers', for all autosomal STR analyzed. The combined paternity index (CPI) obtained was 1 x 10^7 and the probability of paternity (PP) of 99.999%.

The results allowed firstly detecting an infrequent event, such as the paternity of dizygotic twins from different fathers. Secondly they also show that although the frequency of these events is not high, its likelihood is possible, as showed in this study. Therefore, assuming that sometimes it is intended that the same result can be shared by two brothers, by the mere fact of being twins would have meant a great negligence.

360. THE EFFECT OF LINKAGE ON SIBSHIP DETERMINATION BASED ON LIKELIHOOD RATIO

Morimoto C.[1], Manabe S.[1], Kawai C.[1], Fujimoto S.[1], Tamaki K.[1]

[1] *Department of Forensic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan*

In forensic genetics, increasing the number of loci is an efficient strategy of increasing discrimination power. However, with increasing loci numbers, the effect of linkage between the loci may not be negligible in kinship analysis. For example, a recently developed 21 short tandem repeat (STR) typing system (GlobalFiler) has two pairs of linked loci (vWA/D12S391 and CSF1PO/D5S818), which are located within 50 centi-morgans of each other. We investigated the effect of linkage on sibship determination using genotypes of computer-based sibling pairs in the GlobalFiler system.

We computationally generated the genotypes of 10,000 sibling pairs on the basis of Japanese allele frequencies of the 21 loci in GlobalFiler. We included mutational and recombination events while synthesizing these sibling pairs. We calculated two types of LR values, LR_1 considering linkage and LR_2 ignoring linkage for each pair and then compared the values.

The minimum, median and maximum values of LR_1 were 1.6×10^{-4}, 4.3×10^{5}, and 5.4×10^{14}, respectively. These 3 quantiles are similar to those of LR_2 (1.5×10^{-4}, 4.1×10^{5}, and 4.4×10^{14}, respectively). However, approximately 64% of the LR_1 values increased up to 3.9 times. This result suggests that incorporating linkage during LR-based sibship determination is not always conservative. In 35 STR loci including pairs of 7 linked loci, the difference between LR_1 and LR_2 was larger than that in 21 loci. Therefore, we should consider the effect of linkage to calculate rigorous LR values during sibship determination.

361. NON-INVASIVE PRENATAL PATERNITY TESTING: A CASE REPORT

Gysi M., Arora N., Sulzer A., Voegeli P., Kratzer A.

Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

mario.gysi@irm.uzh.ch

The presence of foetal cell-free DNA (cfDNA) in maternal blood has opened

new avenues for non-invasive prenatal paternity testing, thus eliminating the risks of miscarriage associated with invasive methods such as chorionic villus sampling and amniocentesis. Current prenatal non-invasive paternity tests rely on SNP and Y-STR typing because the large excess of maternal cfDNA makes foetal autosomal STR typing very challenging. In this report we describe a case where the prosecution requested non-invasive prenatal paternity testing to investigate the sexual misconduct of a man who committed suicide after allegedly leaving a woman under his supervision pregnant. Foetal cfDNA from maternal blood was analysed by sequencing 317000 SNPs on an Illumina Infinium microarray assay at Natera, inc. (USA), resulting in a paternity probability of 99.9% for the alleged father. Paternity inclusion was confirmed with postnatal STR profiling - with a probability of >99.9999%. While the results are in agreement, the case further reveals that for forensics routine casework, autosomal STR typing from cfDNA would be preferable because STRs are routinely used in forensics and they are associated with well-established biostatistics. With massively parallel sequencing technology entering forensic routine, it is now possible to detect the minor contributor of a DNA mixture at a much smaller proportion and thus this technology offers the possibility to carry out prenatal STR typing. We have started to develop our own massively parallel sequencing protocols to this end, and in addition to the case report, we provide our first findings.

362. MUTATION STUDY OF 28 AUTOSOMAL STR LOCI IN SOUTHWEST CHINESE HAN POPULATION

Jin B.[a, 1, 2], Su Q.[a, 1], Luo H.[1], Li Y.[1], Wu J.[1], Yan J.[1], Hou Y.[1], Liang W.[1], Zhang L.[1]

[1] *Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China*
[2] *Department of Forensic Medicine, North Sichuan Medical College, Nanchong 637000, Sichuan, China*
[a] *These authors contributed equally to this work*

* liangweibo@scu.edu.cn

Short tandem repeats (STRs) are the most world widely used genetic markers in human identity testing. The relatively high mutation rates of STRs have been proved to be disadvantage for both kinship analysis and identification. When calculating the cumulative paternity index (CPI) in parentage testing, mutations should be taken into account. The mutation rates of STRs in Chinese population have been reported previously, but no regional specific information was offered considering the difference due to geographic span. In this study, we focus on the mutation of 13 CODIS and 15 additional STRs, which were widely tested in the southwest Chinese Han population to dedicate reliable information on mutation for forensic practice in this region.

Mutation rates and 95% CI of 28 loci were investigated for almost 100,000 parent(s)-child meiotic transfers obtained from 5569 paternity testing cases. Overall, 169 mutation events were observed. 168 events (99.4%) were single-step mutations, only 1 event (0.59%) was double-step mutation. The average mutation rate was 17.01×10^{-4}. The locus-specific mutation rate varied from 4.39×10^{-4} to 59.12×10^{-4}. Mutation rates varied with the ethnic lines and regions. There was no significant difference between mutation expansion and contraction. Paternal origin mutations occurred more frequently than maternal origin ones. In addition, mutation rates indicated a positive correlation with the Heterozygosity and geometric mean of LRPR, respectively. Short alleles had the trend toward mutation gain while long alleles trended toward mutation loss.

363. AN APPROACH FOR EXCLUSION OF FULL-SIBLING RELATIONSHIP WHEN MULTIPLE KNOWN FULL-SIBLINGS INCLUDED-5TH ALLELE EXCLUSION METHOD

Wang X.[1], Zhang G.[2]

[1] *School of Criminal Investigation, Southwest University of Political Science and Law, Chongqing, China*
[2] *School of Forensic Medicine, Shanxi Medical University, Taiyuan, China*

forensic@163.com

Objective: Establish a method for examination of full-sibling relationship-the 5th allele exclusion method. Method: According to Mendel's genetic law, the total number of alleles on a specific STR site detected from full-sibling's parents should be 1-4. When more than 2 known full-siblings joined in the examination for the full-sibling relationship identification of a suspect, the total number of different allele from all the tested people should be less than 5. If not, the STR was named 5th allele STR and such a STR site is out of the genetic law. If 3 or more 5th allele STRs were detected in an identification system, the suspect should be excluded, i.e. the suspect was not a full-sibling of the known-siblings. To evaluate the exclusion power of STR system, 19 STR loci of 2 or 3 known full-siblings were satisficed and compared with 100-unrelated persons in this study using the Goldeneye™ 20A kit. Results: When 2-known full-sibling participated the examination, the exclusion rate were 47.7% while the corresponding exclusion rate raised to 88.0% when 3-known full-siblings were included, the data came from 100 unrelated DNA typing using the 19 autosome STRs from Goldeneye™ 20A kit. The STR was named efficient STR when the total number from known full-siblings was 3 or more. The exclusion rate rises while the efficient STR number increases. Conclusion: The 5th allele exclusion method is simple and clear. This approach could be used in the full-siblings relationship examination when 2 or more known full-siblings join the full-sibling identification.

1. Tamura T, Osawa M, Kimura R, et al. Evaluation of the Allele-sharing Approach, Known as the IBS Method in Kinship Analysis. Journal of forensic and legal medicine. 2013, 20(2): 112-116.
2. LU Hui-ling, ZHOU Ke-wei, LU De-jian1, et al. Application of ITO Method and Discriminant Functions in Full Sibling and Half Sibling Identification. Journal of Forensic Medicine, 2009, 25(2): 118-122.
3. ZHAO Shu-min, ZHANG Su-hua, QUE Ting-zhi, et al. Establishment of Universal Algorithms for Commonly Used Kinship Indices between Two Individuals. Journal of Forensic Medicine, 2011, 27(5): 330-333.

Theme 15: Forensic Biology

364. FINDING DNA: USING FLUORESCENT IN SITU DETECTION

Haines A. M.[1], Tobe S.[1], Kobus H.[2], Linacre A.[1]

[1] *School of Biological Sciences, Flinders University, Adelaide, Australia*
[2] *School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia*

alicia.haines@flinders.edu.au

It is known that DNA can be deposited onto a surface by touch yet few means have been developed for its *in situ* detection. Collecting touch-DNA samples can be difficult as likely locations rather than the DNA are targeted leading to many samples that are submitted to a forensic laboratory containing little or no DNA. A range of dyes are available that bind to DNA at high specificity for application within the laboratory and here we report on the use of these dyes to detect latent DNA on various substrates and within biological samples. Six common nucleic acid-binding dyes were selected due to their increase in fluorescence when in the presence of double stranded-DNA; four of the six dyes are permeable to cell membranes. The fluorescence from dye/DNA complex was detected using a high intensity light source, the Polilight® (PL500), an excitation wavelength of 490 nm and emission observed/recorded through interference filters centred at 530 nm or 550 nm depending on the dye emission. The samples were visualised under a fluorescent microscope (Nikon Optiphot) using a B2A filter cube. The detection limit of DNA was determined for the selected dyes along with the optimal conditions, such as buffer composition and dye concentration for a range of surfaces. The ability for the dyes to detect DNA within biological samples such as saliva, hair, skin, fingermarks, and hair follicles was also determined. Recommendations as to the optimum dye to detect latent DNA will be provided as a conclusion to this presentation.

365. ON THE EFFECT OF SHOT DISTANCE, BALLISTIC MODEL CONSTRUCTION AND DOPING AND THE TYPE OF GUN ON THE SIMULTANEOUS ANALYSIS OF DNA AND RNA FROM BACKSPATTER RECOVERED FROM INSIDE SURFACES OF FIREARMS

Grabmüller M.[1], Cachée P.[2], Madea B.[1], Schyma C.[3], Courts C.[1]

[1] *Institute of Legal Medicine, University of Bonn, Stiftsplatz 12 Bonn, Germany*
[2] *Cachée Group, Birkenweg 6 Neuseddin, Germany*
[3] *Institute of Legal Medicine, University of Bern, Bühlstrasse 20 Bern, Switzerland*

mgrabmue@uni-bonn.de

When a firearm projectile hits a biological target a spray of biological material (e.g. blood and tissue) can eject from the entry wound and be propelled back into the direction of the firearm ('backspatter'). Traces of backspatter may consolidate on and be recovered from the shooter and the shooter's surroundings but also inside surfaces of the firearm and molecular analysis of backspatter has already shown its potential for DNA based victim identification and activity reconstruction by trace contextualisation via RNA analysis.

Here we assessed whether 'triple contrast' doping of ballistic models interferes with forensic analysis of DNA, mtDNA and co-extracted mRNA and miRNA from backspatter collected from inside parts of firearms generated by experimental shootings. Additionally, we investigated the effect of several combinations of shot distances and types of firearms on backspatter generation and co-extraction and simultaneous analysis of DNA and RNA isolated from backspatter.

We demonstrate that 'triple contrast' stained biological samples collected from inside surfaces of firearms are amenable to forensic DNA profiling and permit analysis of the entire mtDNA D-loop even for 'low template' DNA amounts that preclude standard short tandem repeat DNA analysis. Furthermore, we show the effect of shot distance and the type of firearm in experimental shootings on the yields of DNA and RNA co-extracted from backspatter and the success rates of forensic DNA profiling and RNA based organ identification.

366. SEPARATION PATTERNS OF SPERM MIXTURES: THE MAJOR-MINOR IMPACT

Seah L. H., Wee B. H.

Department of Chemistry Malaysia, Kuching Branch, Lot 3148, Block 14, Jalan Sultan Tengah, 93050 Petra Jaya, Kuching, MALAYSIA

seahlayhong77@gmail.com

Abstract

Differential extraction of semen stains is intended to separate the epithelial DNA from the sperm DNA into the non-sperm and sperm extracts respectively. However, the separation profiles of stains containing entirely of sperm mixtures (as usually encountered in gang-rape) are determined by the proportion of the sperm DNA contributed by the respective sources in the mixtures. Experiments with controlled amounts of sperm mixtures on cloth and FTA cards demonstrated the DNA of the major sperm contributor persisted in the sperm extract and the DNA of the minor sperm contributor(s) is usually detected only in the non-sperm extract. The interpretation of results from differential extraction of semen mixtures particularly in inferring cell-origin thus requires a careful consideration of the derived separation profiles. The relevance of the evidence and the probative value of the DNA profile are important issues in the evaluation of forensic evidence from sperm mixtures.

367. EXTRACTION OF DNA FROM GASTRIC CONTENT IN DIFFERENT STAGES OF DIGESTION

Schwerer M. J.[1], Vogl S.[1], Graw M.[1]

[1] *Institute of Forensic Medicine, Ludwig-Maximilian-University, Munich, Germany*

Michael.Schwerer@med.uni-muenchen.de

Background: The investigation of the gastric content in forensic medicine

can provide valuable information about the final hours of a deceased person. Studying the composition of a last meal, PCR-based techniques are undoubtedly the most sensitive tools. However, molecular techniques crucially rely on the availability of high-quality DNA. The present study compared three different DNA preparation methods for their applicability on digested food. Material and Methods: Gastric content from 48 autopsy cases was investigated. DNA was prepared employing a magnetic beats based system (Promega, Mannheim, Germany) as well as a standard silica column based extraction kit (Qiagen, Hilden, Germany). In addition, a silica column based system specially designed for food surveillance (Qiagen) was applied. The effectiveness of DNA preparation was evaluated using a fluorescence based measurement system (Life technologies, Darmstadt, Germany). Results: Successful DNA preparation was observed from all specimens with all techniques. However, the obtained DNA concentrations varied between 0.1 µg/ ml and 24 µg/ ml between the individual experiments. The outcome of DNA was correlated with the stage of digestion of the gastric content. The magnetic beats based system was most successful for highly digested gastric content, but unsuitable for nearly undigested material. The latter specimens were most effectively processed using the food surveillance system. The standard silica column based system was suitable for material in all stages of digestion, but provided lower rates of DNA outcome. Conclusion: Effective DNA preparation from gastric content requires different extraction techniques based on the degree of digestion of the material.

368. SYSTEMATIC ANALYSIS OF DECADACTYLAR SUBUNGUEAL GENETIC TRACES IN MURDER CASES

Caputo M.[1,2] , Canónaco E.[3], Corach D.[1,2]

[1] Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry Universidad de Buenos Aires, Argentina
[2] Consejo Nacional de Investigaciones Científicas y Tecnológicas-CONICET, Argentina
[3] Cuerpo Médico Forense de la Justicia Nacional, Argentina

dcorach@ffyb.uba.ar

Foreign biological material under the fingernails of a murder victim and/or suspect can be valuable evidence in a criminal investigation. The aim of this study was to evaluate the prevalence of foreign DNA under each fingernail of victims of violent deaths, including fatal struggle or rape cases.

Fingernail swabs or, alternatively clipping nails, were taken from all fingers of both right and left hands from 39 victims (13 females and 26 males) and treated separately. Semiautomated extraction was performed and 21 markers were genotyped.

We detected reliable profiles in all the studied samples (n=390). The majority of results produced a single profile that matched the donor. Foreign DNA was detected in 13/39 victims, 61% positive detection in females vs 24% in males. A total of 32/390 DNA mixtures were detected in biological material under the fingernails being 17 from right hand (53%) and 15 from left hand (47%). The finger-like distribution was similar in right and left hands where the grooming, middle and ring fingers showed the 84% of the positive results; being these findings in line with the strength and shape of hand's fingers.

The sample treatment optimization increased the possibilities of obtaining biological material from the perpetrator being a powerful tool in a murder investigation. Processing strategy including the pentadactylar analysis of each hand for independent subungueal recovery of informative exogenous genetic material by fingernail clipping or swabbing and the use of an optimized extraction and purification protocols might increase the rate of success in identifying criminal felons.

369. ANALYSIS OF DNA FROM FINGERNAIL SAMPLES IN CRIMINAL CASES

Bozzo W., Colussi A., Ortiz M., Laborde L., Canutti L., Pilili J., Carini G., Lojo M. M.

Laboratorio de Análisis Comparativo de ADN, Asesoría Pericial Departamental

La Plata, Dirección General de Asesorías Periciales, Suprema Corte de Justicia de la Provincia de Buenos Aires, Argentina

agcolussi@gmail.com

DNA profiles obtained from the fingernail hyponychium may be important evidence in the investigation of certain criminal acts. The finding of foreign DNA could evidence the transfer of genetic material between the perpetrator and the victim, originated from the assault or to previous contact between them.

We study DNA samples from 164 fingernail samples submitted to our lab in 71 criminal cases. They were obtained by three different collection methods: nail avulsion, clipping or swabbing. In most cases samples were taken during the autopsy operation from homicide cases.

In 74.6% of the analyzed samples an informative genetic profile was obtained. In only the 13.2% of the processed samples a foreign DNA profile was detected as a mixture where the owner profile was the major contributor.

In the present study foreign DNA under fingernails was detected in a low value compared with others authors. This could be because most samples are routinely submitted for DNA analysis without a prior selection of cases based on the exhaustive evaluation of the events produced during the commission of each offense.

370. GENETICS 4N6FLOQSWABS ARE SUPPORTING DNA PRESERVATION FROM BUCCAL SWABS AFTER LONG TERM STORAGE

Squassina A., Castriciano S.

Copan Italia Spa, Brescia Italy

alice.squassina@copanitalia.com

Properly collected and stored samples are important for forensic investigations. Copan developed the Genetics 4N6FLOQSwabs for Forensic Genetics testing of reference and paternity samples. Genetics 4N6FLOQSwabs are available in 3 formats: in peel pouch, dry long tube and short tube with Active Drying System (ADS). The objective of this study was to evaluate the long term storage stability of human DNA from buccal samples collected with Genetics 4N6FLOQSwabs.

Genetics 4N6FLOQSwabs with and without ADS were used to collect buccal samples from 30 donors in 5 replicates. After collection, swabs without ADS were left to dry at room temperature for one hour, while the swabs with ADS were stored in their tubes. Sets of both swabs were analyzed at 0 time, and after 3 and 6 months (12 months is ongoing) at RT and -80°C. After each time point, DNA was extracted from all swab using Prep Filer Express on Automate Express, quantified using Quantifier Trio and profiled using Identifier Plus kit (28 PCR cycles) on 3130 Genetic Analyzer (all by Life Technologies). Complete STR profiles were obtained from all samples collected with both Genetics 4N6FLOQSwabs up to 6 months at RT and -80°C. An average of 50 ng/ul of DNA was recovered from all swabs at T0, and an average of 10 ng/ul DNA with a degradation index of ≤1) after 6 months at RT, and -80°C.

Copan Genetics 4N6FLOQSwabs with and without ADS are suitable for long term preservation of DNA from samples for paternity and forensic investigations.

371. EVALUATION OF COLLECTION PROTOCOLS FOR THE RECOVERY OF BIOLOGICAL SAMPLES FROM CRIME SCENES

Aloraer D., Goodwin W.

[1] School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, United Kingdom

Dbnaloraer@uclan.ac.uk

The main focus in forensic genetics in the past 20 years has been to increase efficiency of the extraction and identification of DNA from a wide variety of evidence, and to improve DNA profiling technology by making it more sensitive and robust. However, the methods used to recover DNA evidence

from crime scenes have seen little development. This study aimed to improve the efficacy of the collection and storage up to the point where the evidential material is received at a laboratory. We have compared the collection of biological evidence with swabs using ultrapure water as a wetting agent with the use of a propriety detergent-based wetting agent. We have found that while the recovery of biological material using the detergent-based wetting agent is marginally better the stability post-collection is greatly improved. When using ultrapure water as the wetting agent DNA degradation can be seen after approximately 6 h at room temperature. Using the detergent-based solution stabilizes DNA for up to 48 h, even when the temperature is increased to 37 °C. The impact of this is likely to be limited in circumstances where crime scene evidence can be kept at low temperatures until it reaches the laboratory; however, in contexts where this is problematic, the modified method for collection could have a large impact on the preservation of forensic evidence before it reaches the laboratory.

372. SEXUAL AGGRESSIONS SAMPLING FOR SEMEN FLUID WITH 4N6FLOQSWABS: WHAT IS THE OUTCOME?

Martínez P.[1], Santiago B.[1], Alcalá B.[1], Lozano B.[2], Miguel L.[3], Godinez U.[4], Ortega G.[5], Vallejo G.[1]

[1] National Institute of Toxicology & Forensic Science José Echegaray 4 -28232 Las Rozas, Madrid, Spain
[2] Forensic Phys. Juzgados 1, 2, 7 Alcorcón, Madrid
[3] Forensic Phys. Juzgado 1 Collado Villalba, Madrid
[4] Forensic Phys. Juzgado Alcalá de Henares, Madrid
[5] Forensic Phys. Institute of Legal Medicine, Guadalajara, Spain

pilarm.fernandez@justicia.es

Semen detection is primordial at the forensic laboratory from evidences coming from a sexual aggression; however, such investigation may be problematic because the fluid may come with low sperm cell concentration[1]. Recently, a three-fold increase of sperm recapture was obtained from nylon *4N6FLOQSwabs*™ (*Copan Flock Technologies*) elution, out of semen controls from normospermic and oligospermic donators[2], in comparison to cotton swabs. In Benschop et al. (2010)[3], vaginal postcoital samples were not from real casework and sperm recapture values were not presented. In our study, sexual assault casework evidences (N>50) were taken alternatively with both nylon and cotton swabs from each victim's body area, and then analyzed for semen searching. The swabbing was sampled by different practitioners so the inter-individual factor was considered. The present work is the first study in real casework evidences taken from *4N6FLOQSwabs*™ where both sperm recapture and semen fluid data comparisons are analyzed and discussed prior genetic quantification.

1. López-Teijón M: Semen quality in a population of volunteers from the province of Barcelona. Reproductive Biomedicine. 2007; 15(4): 434–444.
2. Martínez P, García MG and Vallejo G. Enhanced semen elution from *4N6FLOQSwabs*™ and cotton swabs prior DNA analysis. In: International Symposium on Human Identification; Phoenix, AZ. 29 Sep.-2 Oct. 2014.
3. Benschop CCG, Wiebosch DC, Kloosterman AD and Sijen T: Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. Forensic Science International: Genetics. 2010; 4: 115–121.

373. POTENTIAL DEGRADING EFFECT OF SODIUM HYPOCHLORITE ON EXHIBITS CONTAINING DNA

Szkuta B.[1,2], Ballantyne K. N.[1,3], van Oorschot R. A. H.[1]

[1] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Melbourne, Australia
[2] School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia
[3] School of Psychology and Public Health, La Trobe University, Bundoora, Australia

bszk@deakin.edu.au

In the interest of DNA contamination minimisation, the use of sodium

hypochlorite as a cleaning reagent is common practice among many laboratories. Whilst its effectiveness in the decontamination of tools and surfaces has been verified at specific concentrations, it has not yet been established whether any residual sodium hypochlorite potentially remaining on the tools/surfaces has a detrimental effect if direct contact is made with an exhibit containing DNA. Surfaces were treated with 1% Hypochlorite (air-dried), 10% hypochlorite (air-dried), 10% hypochlorite wiped dry with Kimwipes™, or 1% Hypochlorite wiped dry with Kimwipes™ followed by the application of water (wiped dry), as per standard laboratory procedure. Treated surfaces were contacted with mock-exhibits containing 200ng of DNA (within 100µL). In order to observe the potential degrading effects of sodium hypochlorite, contacted mock-exhibits and untreated control samples of DNA were analysed to determine the quantity and quality of the DNA within the samples. No degrading effect on DNA quantity/quality was observed, with the exception of samples that came into contact with surfaces treated with 10% hypochlorite. It is therefore recommended that surfaces cleaned with high concentrations of hypochlorite be rinsed with in appropriate agent (water) following application.

374. CRIME SCENE DNA SAMPLING BY WET-VACUUM APPLYING M-VAC

Hedman J.[1,2], Ågren J.[3], Ansell R.[1]

[1] *Swedish National Forensic Centre, Linköping, Sweden*
[2] *Applied Microbiology, Lund University, Lund, Sweden*
[3] *Swedish National Veterinary Institute, Uppsala, Sweden*

johannes.hedman@tmb.lth.se

Swabs provide efficient sampling of crime scene stains from surfaces and items for subsequent forensic DNA analysis. However, swabs are not optimal for large dilute stains or absorbing materials such as fabrics. Tape-lifting works well for fabrics, but is not ideal for dried stains. A possible sampling alternative is the M-Vac wet-vacuum instrument (M-Vac Systems Inc.). We have evaluated the M-Vac for sampling of dried stains on inert surfaces and fabrics. M-Vac gave significantly higher DNA concentrations from dried saliva stains on laminated wood, compared with cotton swabs (mean DNA concentrations 1.14 and 0.57 ng/µL, respectively, $p=0.02$). On glass, M-Vac and cotton swabs gave similar DNA concentrations. Additionally, M-Vac retrieved over twice as much DNA from saliva stains on cotton fabric (T-shirt) compared with towels (terry-cloth), showing that the absorption properties of the surface affect wet-vacuum sampling. M-Vac was also applied for retrieving wearer DNA from clothes, enabling generation of complete DNA profiles from denim jeans, leggings and cotton T-shirt. Partial DNA profiles were retrieved from an "offender" pressing his hand against the shoulder of a person wearing a T-shirt. There, the major parts of the resulting mixed DNA profiles were from the wearer/"victim", indicating that M-Vac may not be ideal for sampling of touch DNA from clothes. Wet-vacuum sampling requires a fairly large instrument, trained users and DNA extraction procedures handling large sample volumes. Still, in especially important cases, wet-vacuum could enable sampling of large dried stains that may be difficult to sample with other procedures.

Theme 16: Legal Medicine

375. GENETIC TESTING OF SUDDEN CARDIAC DEATH GENES IN SUDDEN UNEXPECTED DEATHS

Gréen H.[1,2], Krantz P.[3], Grandell I.[1], Green A.[4]

[1] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden
[2] Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden
[3] Department of Forensic Medicine, National Board of Forensic Medicine, Lund, Sweden
[4] Department of Clinical Genetics, Linköping University Hospital, Linköping, Sweden

Henrik.green@rmv.se

Sudden cardiac death (SCD) in young individuals (age < 40) of end up as "autopsy negative" or "sudden unexplained death" (SUD) with no known cause of death. Genetic cardiac disorders such as long QT syndrome, brugada, ARVC etc. may lead to sudden unexplained death and genetic information might be useful for giving an indication to consider SCD in forensic cases. The aim was to investigate if we could use next generation sequencing techniques to screen forensic SUD to identify if genetic screening might be helpful in determining cause of death. We designed a next generation sequencing assay for identification of pathogenic genetic variants in 81 genes associated with SCD. We used HaloPlex target enrichment system (Agilent) for capturing. 66 samples from SUD were referred to us by forensic pathologists in Sweden and sequenced using Haloplex and a MiSeq-sequencer (2x150 bp, Illumina), multiplexing 4-6 samples/run. Variant evaluation was based on previously known pathogenicity in dbSNP and HGMD as well as predictive models. >99% of the target region were covered in most cases, with an average coverage of >1000X. A total of 10 pathogenic genetic variants and 12 probably pathogenic variants were identified in a total of 20 cases (31%). Two samples failed QC. Pathogenic variants in e.g. LDB3, KCNE1, KCNH2, KCNQ1, TTN and TNNC1 were identified. Genetic information in SUD cases can be useful in getting a basis for considering SCD, although the variants needs to be related to the circumstances and other findings of the case.

376. NEXT-GENERATION SEQUENCING OF 68 GENES IN SUDDEN UNEXPLAINED DEATH YOUNG INDIVIDUALS IN FORENSICS

Buscemi L.[1], Alessandrini F.[1], Perna G.[2], Tagliabracci A.[1]

[1] Institute of Legal Medicine, Università Politecnica delle Marche, Ancona, Italy
[2] Dipartimento di Scienze Cardiovascolari, Ospedali Riuniti, Ancona, Italy.

buscemi@univpm.it

The advancement of knowledge in the field of molecular biology has suggested that genetic abnormalities are the leading cause of sudden unexplained death in young people. The molecular autopsy may identify a cause of death in 30% of cases of SUD.

Three forensic SUD cases were studied. 68 genes most frequently associated with SUD were analyzed by NGS on an Ion Torrent PGM instrument.

Case 1: ♀, 13, was found dead in his room. An autopsy was negative. Genetic analysis showed a missense mutation KCNH2 gene (L955V), pathogenic for LQT2 syndrome.

Cause of death: sudden cardiac death in LQT2 syndrome .

Case 2: ♂, 18, who died during a football game. The boy was subjected to annual health checks; in its history there have been two syncopal episodes at 14 years. The autopsy revealed a circumferential subepicardial fibrosis of the left ventricle, with extension to the septum. Genetic analysis has identified a nonsense mutation in MYH7 gene (K1587X).

Cause of death: sudden cardiac death in arrhythmogenic cardiomyopathy with prevalent left ventricular involvement.

Case 3: ♂, 29 years old, died in the course of a diving. The autopsy gave

evidence of an asphyxiation by drowning. A paternal cousin had a SUD at 21 with a sibling suffering from arrhythmogenic cardiomyopathy, bearer of a defibrillator. Genetic analysis revealed a mutation in the gene ABCC9 (V1137I) associated with early repolarization syndrome, linked to increased risk of arrhythmic death.

Cause of death: asphyxiation by drowning in the early repolarization syndrome.

377. BRUGADA SYNDROME GENETIC TESTING: A RELIABLE SANGER SEQUENCING PROTOCOL

Cisana S.[1], Verzeletti A.[1], Vassanelli F.[2], Bontempi L.[2], Cerri N.[1], Cortellini V.[1], Curnis A.[2], De Ferrari F.[1]

[1] University of Brescia – Department of Medical and Surgical Specialties, Radiological Sciences and Public Health- Forensic Medicine Unit - Brescia, Italy
[2] Department of Cardiology, Spedali Civili, University of Brescia, Brescia, Italy

s.cisana@unibs.it

The Brugada syndrome (BrS) is a potentially lethal, inheritable arrhythmia syndrome affecting about 5 in 10000 people worldwide. This cardiac disorder, triggered by mutations in SCN5A gene (encoding for cardiac sodium channels), results in changes in the onset or propagation of cardiac action potential that can generate arrhythmias, potentially fatal. The relationship between the data obtained through endocavitary electrophysiological study performed in patients with suspected Brugada syndrome and genetic testing can be useful to prevent sudden cardiac death. These data allow a better diagnostic analysis of Brugada syndrome, with direct implications in therapy regarding the opportunity to implant lifesaving devices.

In the last few years, the advent of next generation sequencing (NGS) revolutionized the approach to genetic studies, making whole-genome sequencing a possible way of obtaining global genomic information. However this strategy of sequencing is still in development and it requires in any case capillary electrophoresis data validation. High data quality and accuracy are recognized characteristics of Sanger sequencing analysis.

In this study we developed a Sanger sequencing workflow for SCN5A gene exons involved in Brugada Syndrome. All exons (exon 2 - exon 28) were amplified by PCR and directly sequenced using an ABI PRISM® 310 Genetic Analyzer. Then we optimized the protocol analysis using the kits currently available, which have greater reliability, sensitivity and specificity than in the past.

1. Remme CA: Cardiac sodium channelopathy associated with SCN5A mutation: electrophysiological, molecular and genetic aspects. J Physiol. 2013; 591.17:4099-116.
2. Hofman N, Tan HL, Alders M, Kolder I, De Haij S, Mannens MMAM, Lombardi MP, Deprez RHL, Van Langen I, Wilde AAM: Yeald of molecular and clinical testing of arrhythmia syndromes. Report of 15 years' experience. Circulation. 2013; 128:1513-21.
3. Park HS, Kim YN, Lee YS, Jung BC, Lee SH, Shin DG, Cho Y, Bae MH, Han SM, Lee MH: Genetic Analysis of SCN5A in Korean Patients Associated with Atrioventricular Conduction Block. Genomics Inform. 2012;10(2):110-16.

378. EFFECT OF RNA INTEGRITY ON REFERENCE GENE EXPRESSION STABILITIES IN HUMAN POST-MORTEM WOUNDED AND NORMAL SKIN

Liapi E.[1], Rothschild M. A.[1], Schneider P. M.[1], Gomes I.[1]

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany

eleniliapis@hotmail.com

Wound age estimation focuses on characteristic time-dependent gene expressions during wound healing processes according to studies using animal experimental models. Efforts to evaluate these findings require accurate normalisation particularly for human post-mortem material. In this study, total RNA was extracted from human post-mortem wounded and non-injured (reference) skin tissue. RNA Integrity Numbers (RINs) were obtained

and ranged from 2.2 to 7.8 (mean 4.2±1.8). RNA samples were subdivided into two subgroups within the reference and wound groups according to the extent of degradation (moderate RIN≥4 and ≤7.8; extensive RIN<4). Both moderately and extensively degraded groups were tested by analysing the gene expression of four endogenous control genes: G*APDH, PGK1, PPIA and YHWAZ*. Notably, the subdivision based on RNA integrity affected the gene expression stabilities (M values) assessed by the reference gene validation software, geNorm (Vandesompele et al., 2002). In the moderately degraded reference samples, three genes yielded high expression stability (M<1). Conversely, the extensively degraded reference samples showed reduced expression stability (M>1.5). Interestingly, *PPIA* remained the most stable gene in both degraded categories in the uninjured tissue group. For wounded tissue, higher RNA degradation resulted only in reduced stability (higher *M* values) of all reference genes, without changes in gene stability order. To conclude, as expected, higher RNA integrity across the tested samples resulted in higher reference gene expression stability and vice-versa. The results of this study highlight the importance of RNA integrity research when addressing the normalisation of target genes involved in the molecular response during wound healing.

379. DETECTION OF MIRNAS IN TISSUE SAMPLES AT AUTOPSY: A TRIAL FOR DIAGNOSTICS AT POSTMORTEM

Kakimoto Y.[1], Kamiguchi H.[2], Ochiai E.[1], Satoh F.[1], Osawa M.[1]

[1] *Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan*
[2] *Support Center for Medical Research and Education, Tokai University, Isehara, Kanagawa, Japan*

kakimoto@tokai-u.jp

MiRNAs are single-stranded noncoding RNAs comprised of 18 to 24 nucleotides, which regulate various biological processes at the post-transcriptional level. In contrast to vulnerable mRNAs, miRNAs scarcely suffer from degeneration in the environments, such as severe changes of pH and temperature as well as repeated ice/thaw cycles [1, 2]. This robustness of miRNA should be fitted for postmortem investigations. Here, we utilized stored samples to establish a method to evaluate the miRNA profiles of autoptic tissues exposed to degenerative conditions. Electropherogram showed longer RNAs were fragmented in the long-term fixed samples. Quantitative PCR was performed for seven miRNA species and three other small RNAs in order to determine the appropriate controls for our postmortem analysis. It resulted in that miR-191 and miR-26b were more suitable than the other types of small RNA molecules as they were stably expressed after death and fixation. Further, the quantitation method was applied, using these endogenous controls, to evaluate the expression of previously identified miRNA biomarkers for myocardial infarction, miR-1, miR-208b, and miR-499a. The relative level of miR-499a was noted to significant decrease in patients' tissues (2.1 fold). This study demonstrates the stability of miRNAs in forensic sampling followed by long-term fixation, and the reliable biomarkers during postmortem examination.

1. P Mitchell, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci. 2008;105: 10513-10518.
2. X Chen, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008;18: 997-1006

380. ANALYSIS OF DNA METHYLATION PATTERNS IN SUDDEN INFANT DEATH SYNDROME

Schwender K.[1], Vennemann M. M.[1], Schürenkamp M.[1], Sibbing U.[1], Pfeiffer H.[1], Vennemann M.[1]

[1] *Institute of Legal Medicine, University of Münster, Münster, Germany*

Kristina.Schwender@ukmuenster.de

Sudden infant death syndrome (SIDS) is still the leading cause of death in

infancy after the neonatal period in western countries. One of the main risk factors was found to be prenatal exposure to tobacco smoke [1].

Genetic studies led to the assumption of heritability in SIDS but no specific genes could be identified yet. There is some doubt that SIDS has a clear genetic cause [2]; however, there is some evidence of heritability in SIDS. Consequently, the possible influences of various epigenetic factors are currently discussed as possible influencing factors in the development of SIDS.

Several studies revealed that current and prenatal exposures to cigarette smoke affect global and gene-specific DNA methylation [3]. This leads to the hypothesis that DNA methylation might be the missing link between SIDS and cigarette smoking. To test this, we examined well characterized gene loci, shown to be differentially methylated between infants of smoking and non-smoking mothers. The identified genes are involved in developmental processes, regulation of cell growth and cell differentiation, detoxification of compounds from tobacco smoke, insulin signaling, or development of hematopoiesis [4].

Methylation patterns of these gene loci were investigated in defined SIDS cases of the large German study on sudden infant death (GeSID), a multi-centre case-control study. Results were compared to unaffected control cases.

Initial results of this study will be presented and discussed.

[1] Vennemann MM, Findeisen M, Butterfass-Bahloul T, Jorch G, Brinkmann B, Köpcke W, Bajanowski T, Mitchell EA & GeSID Group: Modifiable risk factors for SIDS in Germany: results of GeSID. Acta Paediatr. 2005; 94(6): 655-60

[2] Opdal SH, Rognum TO: The sudden infant death syndrome gene: Does it exist? Pediatrics. 2004; 114(4): 506-12

[3] Lee KW, Pausova Z: Cigarette smoking and DNA methylation. Front Genet. 2013; 4: 132

[4] Joubert BR, Håberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, Huang Z, Hoyo C, Midttun Ø, Cupul-Uicab LA, Ueland PM, Wu MC, Nystad W, Bell DA, Peddada SD, London SJ: 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect. 2012; 120(10): 1425-31

381. ESTIMATION OF THE TIME OF DEATH THROUGH CLOCK MIRNA EXPRESSION ANALYSIS IN VITREOUS HUMOR

Corradini B.[1], Alu' M.[1], Radheshi E.[1], Gabbolini V.[1], Ferrari F.[1], Santunione A. L.[1], Silingardi E.[1]

[1] Department of Diagnostic and Clinic Medicine and Public Health, Section of Legal Medicine, University of Modena and Reggio Emilia, Italy

beatrice.corradini@unimore.it

The accurate knowledge of physiological and environmental circumstances at death such as the time of the day (daytime/nighttime) when death occurred has a great medico-legal significance. Although forensic pathologists hold several assessment methods based on death-dependent visual and measurable body modifications, a concordance has not been reached yet. Moreover, those available are reliable in the early postmortem period as well as influenced by many confounding factors.

The ever deeper understanding of the molecular mechanisms underlying the light-dependent expression of core genes of the circadian system in mammals, rises the intuition to relate this oscillation with the time at which the biological clock stopped in a dead body. MicroRNAs have a key regulatory role in this process and their tested potentiality for forensics made them investigable biomarkers for this purpose.

Starting from a previous pilot study, we analyzed the expression of 10 miRNA related to circadian genes with a quantitative real-time PCR (qRT-PCR) assay from nearly one hundred vitreous humor samples of Italian subjects died at daytime and at nighttime. Different sampling sessions were planned at increasing postmortem intervals to evaluate clock miRNA stability. The concordance between the molecular assay and classical measures was also

tested.

Preliminary findings support the vitreous humor as a stable starting source for forensic molecular studies and although at an embryonic stage, the analysis of the expression pattern of circadian miRNA may be helpful to establish environmental conditions in postmortem investigations and it could be ideally expanded to other biological matrices.

1. Muggenthaler H, Sinicina I, Hubig M et al: Database of post-mortem rectal cooling cases under strictly controlled conditions: a useful tool in death time estimation. Int J Legal Med. 2012; 126(1): 79-87
2. Mohawk JA, Green CB and Takahashi JS: Central and peripheral circadian clock in mammals. Annu Rev Neurosci. 2012; 35: 445-462
3. Odriozola A, Riancho JA, de la Vega R et al: miRNA analysis in vitreous humor to determine the time of death: a proof-of-concept pilot study. Int J Legal Med. 2013; 127:573 – 578
4. Lech K, Ackermann K, Wollstein A et al: Assessing the suitability of miRNA-142-5p and miRNA-541 for bloodstain deposition timing. Forensic Sci Int Genet. 2014; 12:181-184
5. Maeda H, Ishikawa T and Michiue T: Forensic molecular pathology: its impacts on routine work, education and training. Leg Med (Tokyo). 2014; 16(2):61-69

382. BACTERIAL DIVERSITY IN BERCAEA CRUENTATA GUT DESCRIBED USING NEXT GENERATION SEQUENCING

Long L.[1], Guo J.[1], Li P.[1], Guo Y.[1]

[1] *Department of Forensic Science, Central South University, Changsha, China*

gdy82@126.com

The investigation of postmortem interval (PMI) is the main and important course for forensic examination. Accurate PMI consists of precolonization interval (pre-CI) and postcolonization interval (post-CI). According to the growth pattern and development characteristics of sarecosaphagous insects, we are allowed to judge insects' development stage and calculate the time of each stage experienced for PMI estimation (actually for post-CI estimation). However, the estimation of pre-CI is still an unsolved challenge. Since bacteria play a critical role in attracting insects to cadavers, it is essential for forensic scientists to understand the interactivation between bacteria and insects for pre-CI estimation use, deeply. This study aimed to investigate gut bacteria diversity of *Bercaea cruentata*. The gut microflora were collected from adult and third instars larvae of B. *cruentata*, then we used combination methods of culture-independent manner and DNA technologies (high-throughput sequencing of 16S rRNA amplification on Illumina MiSeq platform) to catalogue the bacterial community. In biochemical tests, 40 species of bacteria were identified. Using high-throughput sequencing, operational taxonomic units (OTUs) were of higher abundance in adult group, and *Firmicutes* and *Proteobacteria* were both predominated in lavae and adult groups. Our study provided data on gut bacteria diversity of B. *cruentata* for the first time. Bacterial community in adult B. *cruentata* gut were observed different taxon richness and relative abundance patterns compared with the larvae gut, but both adult and larvae have preferences for specific bacteria species. Further studies are required to verify the signaling mechanism for bacterial attraction of sarecosaphagous insects in pre-CI.

383. BACTERIAL COMMUNITY SUCCESSION ANALYSIS BY NEXT GENERATION SEQUENCING IN CHANGSHA CITY, CHINA

Guo J.[1], Liao H.[1], Fu X.[1], Zha L.[1], Liu J.[2], Cai J.[1]

[1] *Department of Forensic Science, Central South University, Changsha, China*
[2] *Department of Internal Medicine, Affiliated Hospital of Inner Mongolia University for Nationalities, Tongliao, China*

cjf_jifeng@163.com

The estimation of postmortem interval (PMI) is one of the most difficult tasks in forensic practice, especially in putrefied bodies. After death, organisms are decomposed by a variety of enzymes and microorganisms. To investigate the succession of bacterial community in the decomposition process, rat remains were placed indoor to isolate sarcosaphagous insect interference controlled with a natural group outside in Changsha city, China. Bacterial communities from two regions (buccal cavity and rectum) were sampled when experiment animals were alive, soon after they died and at various time span after death. Bacterial samples were analyzed by high throughput metagenomic sequencing of 16S rRNA gene conducted on an Illumina MiSeq platform. Our data showed that several bacteria genus were potentially useful for estimating the PMI, such as *Proteus, Streptococcus, Ignatzschineria, Acinetobacter, Prevotella and Proteus*. There were significant bacterial community structure differences in taxon richness and relative abundance patterns through the decomposition process and across different body sites. As decomposition progressed, a negative linear relationship for taxon richness was found along with a shift from aerobic bacteria to anaerobic bacteria. Particularly, the arrive and oviposition of sarcosaphagous insects had no obvious influence on bacterial taxa, but accelerated the process of bacterial community succession. We first reported the bacterial biodiversity in the decomposition process in Chinese terrestrial scenarios and climatic conditions. Bacteria have a remarkable potential for estimate the PMI and next generation sequencing is a novel method to support the application of bacteria in forensic science.

384. THE POTENTIAL USE OF FUNGI COMMUNITY IN POSTMORTEM INTERVAL ESTIMATION

Fu X.[1], Guo J.[1], Liao H.[1], Long L.[1], Zha L.[1], Cai J.[1]

[1] *Department of Forensic Science, Central South University, Changsha, China*

cjf_jifeng@163.com

Estimation of postmortem interval (PMI) with fair accuracy is a critical step in death investigations. Although many approaches are available to estimate PMI through physical findings and biochemical tests, accurate PMI calculation by these conventional methods is still difficult because it is readily affected by surrounding conditions, such as ambient temperature and humidity. It is reported that body provides a residence for diverse commensal microbiota. Microorganisms such as fungi have major roles in this microbial community stability and postputrefaction fungi have been recorded repeatedly in association with decomposed mammalian cadavers in disparate regions of the world. The succession and diversity of these fungi are reviewed briefly with a view to their potential as a forensic tool. This application of mycology is an interface to forensic investigation and may provide a means to estimate PMI within serious decomposition. To evaluate the use of succession and diversity of fungi species for PMI estimation, we investigated the Internal Transcribed Spacer (ITS) of fungi community on certain points of time of rat carcasses conducted by Illumina MiSeq platform. Through high-throughput sequencing, several fungi genus such as *Aspergillus, Ophiocordyceps* are found in community succession and the community structure of each sample significantly differs in taxon richness and relative abundance patterns within decomposition progress. The succession and diversity of fungi community in corpse decomposition revealed by the remarkable indicator of ITS would be a potential forensic tool for PMI estimation.

385. THE COMPREHENSIVE ANALYSIS OF MICRORNA IN HEARTS OF STRESS MODEL RAT

Hashiyada M.[1], Hosoya T.[2], Akane A.[1], Matsumoto T.[1], Yoshimura S.[1], Tokiyasu T.[1], Funayama M.[2]

[1] *Department of Legal Medicine, Kansai Medical University, Hirakata, Japan*
[2] *Department of Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan*

hashiyam@hirakata.kmu.ac.jp

Introduction and aim

Non-coding microRNA (miRNA) can be used as a postmortem biomarker to

quantify the physical stress applied before the death. In this study, rats were used as animal models of excited delirium syndrome (EDS), and the expression of miRNAs in the rat hearts were analyzed using the next generation sequencing (NGS) system.

Material and methods

The group A of Sprague-Dawley rats (10 weeks-old, n=3) were given 3.8 g/kg of 21.0 % ethanol using intragastric gavage, and after 30 min, made run on a treadmill at 35 cm/s for 90 min. The group B rats (n=3) were given the same stress, and suppressed with 2 kg water bags for 90 min. Control rats (n=2) were without any stress. Rats were then euthanized by overanesthesia, and hearts were taken into RNA stabilization solution with the approval of the Institutional Animal Care and Use Committee, Tohoku University. Total RNA in the homogenate of 20 mg left ventricle was isolated using a miRNeasy Mini Kit, and "small RNA library" and templates were prepared for the NGS analysis with Ion PGM system and Ion 318 Chip. The obtained data were analyzed by Torrent Suite software with reference to the rat miRNA databases.

Results and discussion

7 miRNAs (miR-126a-3p, miR-145-5p, miR-26a-5p, miR-27a-3p, miR-24-3p, miR-143-3p and miR-16-5p) expressed in groups A and B significantly higher than in control. The miR-16-5p level in group B was higher than that in group A. These miRNAs, especially miR-16-5p, might reflect the physical stress on rat hearts.

386. GENETIC VARIANTS IN UGT GENES

Glaub A.[1], Schneider J.[1], Schürenkamp J.[1], Gasse A.[1], Schürenkamp M.[1], Sibbing U.[1], Pfeiffer H.[1], Vennemann M.[1]

[1] *Institute for Forensic Molecular genetic, University of Münster, Münster, Germany*

Alina.Glaub@ukmuenster.de

Cannabis is one of the most commonly consumed drugs worldwide. A current discussion of legalization in many countries and use of cannabis based medication for various conditions, such as chronic pain, lead to the questions of the risks and benefits of consuming tetrahydrocannabinol (THC). Consequently, a detailed knowledge of the exact THC metabolism is vital to allow informed scientific and societal discussions. The metabolism of cannabis is separated into two phases (1). Whilst in phase I the oxidation of the active metabolite THC takes place, phase II comprises the glucuronidation of the inactive metabolite. Glucuronidation is activated by UGT (UDP-glucuronosyltransferase) enzymes (2). There are different isoforms known for UGT family members (3). For our study the isoforms UGT1A1 and UGT1A3 are of interest. It is well known that genetic variations in the coding sequence can lead to increased or decreased enzyme activity (4); however, the exact correlation between different genetic sequences of the UGT genes and cannabis metabolism is still unknown. In this study we developed a method to detect single nucleotide polymorphisms (SNPs) in the coding sequence of various UGT genes. Test persons who are known to consume cannabis gave informed consent for genetic analysis and screening of THC and its metabolites. Initial results of this study will be presented and discussed.

(1) Mazur A, Lichti CF, Panther PL, Zielinska AK, Bratton SM, Gallus-Zawade A, Finel M, Miller GP, Radomińska-Pandya A and Moran JH: Characterization of human hepatic and extrahepatic UDP-glucuronosyltransferase enzymes involved in the metabolism of classic cannabinoids. Drug metabolism and disposition. 2009; 37: 1496 – 1504

(2) Mackenzie PI, Miner JO and McKinnon RA: Polymorphisms in UDP glucuronosyltransferase genes: functional consequences and clinical relevance. Clinical chemistry and laboratory medicine, 2000; 38: 889 - 892

(3) Stingl JC, Bartels H, Viviani R, Lehmann ML and Brockmöller J: Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic review. Pharmacology& Therapeutics, 2014; 141: 92 – 116

(4) Chen Y, Chen S, Li X, Wang X and Zeng S: Genetic variants of human

UGT1A3: Functional characterization and frequency distribution in a Chinese han population. Drug metabolism and disposition, 2006; 34: 1462 - 1467

Theme 17: Non Human DNA

387. RESIDUAL SOIL DNA EXTRACTION IMPROVES BIOTA CHARACTERIZATION FOR FORENSIC DNA ANALYSIS

Young J. M., Weyrich L. S., Austin J. J., Cooper A.

Australian Centre for Ancient DNA, University of Adelaide, South Australia, 5005, Australia

High-throughput sequencing (HTS) allows rapid characterisation of soil communities, providing biodiversity information unique to a particular location. However, inefficient DNA extraction can markedly alter the measured abundance of taxa, or prevent some taxa from being detected altogether. For forensic soil discrimination, isolation of the least ubiquitous taxa is desirable to maximize resolution between samples. Here, we compare fungal DNA profiles obtained from a standard commercial DNA extraction kit to three modified protocols: an additional 24-h lysis incubation step at room temperature; 24-h lysis incubation step at 55°C; and re-extraction of the residual soil pellet. Most notably, we found that re-extraction of the residual soil pellet offers a novel tool to enhance resolution between soils. To further examine soil re-extraction, we characterized eukaryote diversity from three subsequent re-extractions and explored the impact of different bead types and lysis methods (vortex or bead beating). Although DNA profiles varied between subsequent extracts, the lysis method (vortex or bead beating) had a significant impact on the DNA profile observed, predominately affecting the proportion of fungi detected. We then applied a similar re-extraction approach to New Zealand volcanic ash sediments, including layers dated to 15,000 years old, which had previously failed to yield DNA. We successfully obtained DNA profiles from these sediments demonstrating the benefit of re-extractions to samples with low DNA quantity and quality. Overall, residual soil DNA extraction offers a tool to maximise yield, diversity and discriminatory power from soil samples typical in forensic casework.

388. A NOVEL FORENSIC DNA PROFILING TECHNIQUE FOR PROTECTED SPECIES

Ciavaglia S.[1,2], Tobe S.[1], Donnellan S.[3], Henry J.[2], Linacre A.[1]

[1] School of Biological Sciences, Flinders University, Bedford Park, South Australia, Australia
[2] Forensic Science SA, Adelaide, South Australia, Australia
[3] South Australian Museum, Adelaide, South Australia, Australia

sherryn.ciavaglia@flinders.edu.au

The intensifying issue of global wildlife crime is illustrated by the 2015 World Wildlife Day slogan 'It's time to get serious about wildlife crime'. To combat illegal wildlife trade, we have developed a novel forensic STR typing panel of three 11-plex assays, examining 28 tetra- and penta-nucleotide repeat loci, for the Australasian carpet python (*Morelia spilota*). The illegal wildlife trade has been valued between US$7-23 billion per annum and is now acknowledged as a significant conservation and global security threat by world leaders. Forensic techniques are required to compliment regulatory and enforcement initiatives. Despite inclusion in CITES Appendix II, native carpet pythons are unlawfully taken from the wild, destined for illegal international trade. The Australian carpet python provides a model species to demonstrate the development and application of forensic genetic techniques to enforcement and investigation of criminal activities involving wildlife. The STR panel is undergoing validation for application to forensic casework. Over 150 alleles have been sequenced and allelic ladders implemented to ensure correct genotyping. Loci have been examined for polymorphic content, heterozygosity and linkage equilibrium. An allelic frequency database has been constructed using over 200 native carpet pythons spanning the species range that can provide probabilistic answers to inquiries of relatedness and geographic origin. Allele frequencies are used to examine population substructure, inbreeding, probability of identity and probability of paternity; the database also has potential to help predict the geographic provenance of a poached python. This STR panel will provide robust and reliable evidence in future wildlife criminal investigations.

389. IDENTIFICATION OF DOG SALIVA USING THE MRNA EXPRESSION ASSAY

Nakanishi H.[1,2], Hara M.[2], Takahashi S.[2], Takada A.[2], Saito K.[1,2]

[1] Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan
[2] Department of Forensic Medicine, Saitama Medical University, Saitama, Japan

hnakani@juntendo.ac.jp

Introduction: When a crime involves a dog bite, identification of dog saliva is evidentially important. Human saliva is identified via detection of α-amylase, but this test cannot be used to identify dog saliva because dogs have low levels of α-amylase. Therefore, we explored whether dog saliva could be identified by detection of dog saliva-specific mRNAs.

Materials and Methods: Canine saliva swabs (n=20), urine swabs (n=20), body surface swabs (n=20), and whole blood samples (n=10) were tested. We analyzed the expression levels of the genes encoding statherin (STATH), carbonic anhydrase VI (CA6), and dog allergens (Canf1 and Canf2) as saliva-specific genes; and the levels of genes encoding β-glucuronidase (GUSB), hypoxanthine phosphoribosyltransferase 1 (HPRT1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping genes. mRNA expression levels were quantitated via real-time PCR using the TaqMan system. If a Ct difference between a target and housekeeping gene was evident within 40 cycles, the mRNA expression level of the test gene was defined as positive.

Results and Discussion: *GAPDH* mRNA served as the control housekeeping gene because *GAPDH* mRNA was readily detected in all samples. The detection proportions of *STATH*, *CA6*, *Canf1*, and *Canf2* mRNAs in saliva samples were 19/20, 1/20, 11/20, and 4/20, respectively. *STATH* mRNA was detected in one body surface sample, but not in any urine or blood sample, suggesting that *STATH* evaluation is optimal for identification of dog saliva.

390. THE SPECIES SPECIFIC OF 13 MICRORNAS IN ANIMAL SALIVA

Peng D.[1], Li Z.[1], Wang L.[1], Su Q.[1], Jiang Y.[1], Zhu J.[1], Wang H.[2], Mao J.[2], Liang W.[1], Zhang L.[1]

[1] Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China
[2] Department of Forensic Genetics, Institute of Forensic Science, Chengdu Public Security Bureau, Chengdu 610081, Sichuan, China

liangweibo@scu.edu.cn

MicroRNAs (miRNAs) have been proved exist in forensically relevant body fluids, However, we need to know whether the result would be influenced by other species contamination or not. This study aims to analyze the expression level of the 13 miRNAs mentioned above in different species, including homo sapiens, felis catus, canis lupus familiaris, cavia porcellus, sus scrofa, mus musculus, oryctolagus cuniculus, bos taurus, capra hircus. MiRNA was extracted by Qiagen kits from 9 kinds of saliva of different species. Reverse transcription and quantitive PCR performed through SYBRGreen method following the protocol with water as negtive control and U6snRNA as reference. Some of target miRNAs were found high expressing in different body fluids of both human and animals. MiR-16 which considered high expressed in blood by Erin K. Hanson et al. expresses high in 5 species saliva, miR-200C which high expresses in human being saliva by Cornelius Courts et al. also has a high level of expression in 3 species. Our results suggest that the species experiments should be analyzed before identify the stains found in crime scene because the stains may be contaminated by some other species. We propose to carry on species specific research when seek a new candidate miRNA marker for forensically relevant human fluids.

393. THE PRESENCE OF DIPTERA LARVAE IN HUMAN BONES

Skowronek R.[1], Tomsia M.[1], Szpila K.[2], Droździok K.[1], Kabiesz J.[1]

[1] Department of Forensic Medicine and Forensic Toxicology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland
[2] Chair of Ecology and Biogeography, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Toruń, Poland

rafal-skowronek@wp.pl

The presence of Diptera larvae in the bone marrow cavities so far has been the subject of very few scientific papers. The Japanese authors found the 3rd instar larvae of the family Piophilidae (Diptera) in human skeletal remains (marrow space of the right femur) during a forensic autopsy [1]. In turn, German authors discovered larvae of *Stearibia nigriceps* and *Liopiophila varipes* (Diptera, Piophilidae) within the medullary cavity of deer bones (tibia, metacarpus, humerus) during a forensic entomological and anthropological field study [2]. The aim of this study is to present our own experiences with the presence of larvae in human bones sent to our Department, mainly by the prosecutors, during criminal investigations. The results of morphological examination of the bones and entomological analysis of discovered larvae were presented. We found numerous larvae of *Stearibia nigriceps* and surprisingly single third instar larva of *Lucilia sericata* and puparium of *Sarcophaga argyrostoma*. We confirmed that larvae are able to penetrate the bones of human corpse. Forensic geneticists may find such valuable entomological evidence (hidden larvae population) during routine preparation of the bones before DNA extraction procedure. In this case, the larvae should be always preserved for further possible forensic entomological studies.

1. Kirinoki M, Hitosugi M, Chigusa Y, Kurosu A and Tokudome S: Larvae of the family Piophilidae found in the marrow space of skeletal remains during a forensic autopsy. Medical Entomology and Zoology 2010; 61: 115–119
2. Reckel F, Hoke N, Heurich N and Grunwald J: Piophilid maggots found

inside of bones. 9th Meeting of the European Association for Forensic Entomology 2012; 88

394. ADVANCES IN SOIL SAMPLE NGS DATA MANAGEMENT FOR FORENSIC INVESTIGATION

Põlajev A., Simm J., Aaspõllu A.

Tallinn University of Technology, Tallinn, Estonia

anu.aaspollu@gmail.com

DNA forensics still has astonishing applications in criminalistics. In addition to human DNA identification and databasing, a soil DNA analysis is a valuable resource among others in forensic investigation. Classical soil analysis has certain limitations. For that reason, a microbial DNA profiling using next-generation sequencing (NGS) has been proposed.

The aim of the present study is to continue our previous work on the elaboration of criteria for soil metagenome data management and database searching, and to test our prior hypothesis.

Therefore, in addition to our previous dataset, which consisted of 11 sample collections from diverse environments, we included new sample sets. Some of these new sample collections were collected from locations that are in close proximity to sampling areas used earlier. Altogether we currently used 11 sample collections. As earlier, the single sample collection consisted of 9 soil samples per one sampling area, spaced by 15 m. In our preceding research we have shown that eukaryotic 18S RNA gene V2-V3 region could be used for efficient data management and database handling, so we have currently focused on that marker region. The data analysis was performed using NCBI BLASTN analysis and custom-made programs in the R language. Preliminary filtration of sequences was based on primer sequences. The selected sequences searched for matches in the NCBI nucleotide database. The matches are then used for sample comparisons to reveal similarities and differences between sampling areas.

In conclusion, our ideas could fill some gaps in the field of forensic soil identification.

395. A STUDY OF ONE SOIL, ITS RELATIVES AND CONTAMINANT'S BY ARBITRARY PRIMED PCR WITH 50MER BASED ANALYSIS

Burgoyne L.[1], Koh L.[1], Catcheside D.[1]

[1] School of Biological Sciences, Flinders University, Adelaide,South Australia, 5001

leigh.burgoyne@flinders.edu.au

A single suburban roadside-strip-soil has been taken as a model for a study to answer questions about the meaning of the concept of "locality" in suburban soils using DNA-based examination methods designed to have no prior-expectations about what DNA may be present. The soil was sampled over three years at two seasons and at sites a metre apart. Sequences unique to the site with respect to a total of another 17 sites were extracted and examined.

396. ANAYSIS OF MTDNA CONTROL REGION FROM SINGLE CAT HAIRS

Wesselink M.[1,2], Bergwerff L.[1], Kuiper I.[1]

[1] Netherlands Forensic Institute, The Hague, The Netherlands
[2] Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

m.wesselink@nfi.minvenj.nl

Different parts of feline mitochondrial control region have been described as forensically relevant. The most frequently described region (between repeats RS2 and RS3) has proven insufficient to distinguish between the majority of random bred cats in The Netherlands[1]. Additional analysis of the 5'region (prior to RS2) decreased the random match probability in this study from 0.47 to 0.29.

Amplification of these two regions in a single reaction, includes amplification of the RS2 repeat which can vary between 180 and 600 bp. Especially when single shed hairs are being investigated, amplification of this 1.2 - 1.6 kb fragment may fail. To circumvent amplification of the repeat without drastically decreasing the number of potentially informative nucleotide positions that are obtained, a new amplification and sequencing strategy was designed.

The PCR and sequencing strategy, its success on single cat hairs and a case example will be presented.

1. Wesselink M, Bergwerff L, Hoogmoed D, Kloosterman AD, and Kuiper I: Forensic utility of the feline mitochondrial control region - A Dutch perspective. Forensic Science International: Genetics. 2015; 17:25-32

397. DEVELOPMENT OF FORENSICALLY INFORMATIVE DNA MARKERS FOR SHORT-BEAKED ECHIDNA – THEIR UTILITY IN PEDIGREE TESTING AND APPLICATION IN WILDLIFE TRADE

Summerell A.[1], Johnson R.[2], Gunn P.[1], Roux C.[1], Frankham G.[2]

[1] Centre for Forensic Science, University of Technology Sydney, Sydney, Australia
[2] Australian Centre for Wildlife Genomics, Australian Museum, Sydney, Australia

alex.summerell@gmail.com

Validation of true genetic pedigree in captive bred animals is only possible through the development and calibration of highly variable species-specific DNA markers[1]. The Short-beaked Echidna (Tachyglossus aculeatus) is a significant species to the zoo industry as an iconic Australian animal of conservation importance, and similarly as a monotreme that is very difficult to breed in captivity[2]. Captive bred animals are in high demand as a consequence, as some aspects of their captive care and husbandry are still poorly understood.

According to international data, there is an increasing trend for wild-caught animals being laundered as 'captive bred' animals[3]. These animals are often offered with paperwork supporting their 'captive bred' pedigree but this paperwork does not contain any genetic data for Zoo and Aquarium Association industry members to make an informed decision upon. There are already precedents for this in the domestic dog, cat and equine industries, and similar research has commenced for wild animals such as the rhinoceros and some species of snakes. This project is developing such a program for an important Australian species that is increasingly being imported to other countries as captive bred3. This collaborative project is drawing upon existing echidna genetic data to develop highly variable genetic markers which will be evaluated and selected to determine relatedness, using informative SNP (Single Nucleotide Polymorphism) markers. The SNPs are being tested upon samples taken from echidnas of known pedigree sourced via project collaborators Perth Zoo and Currumbin Sanctuary. This project is in collaboration with the Wildlife Forensic Unit at the Australian Museum Research Institute.

1. Sunnucks P: Efficient genetic markers for population biology. Trends in Ecology and Evolution. 2000; Volume 15: page 199 – page 203
2. Ferguson A and Turner B. Reproductive parameters and behavior of captive short-beaked echidna (Tachyglossus aculeatus acanthion) at Perth Zoo. Australian Mammology. 2013; Volume 35: page 84 – page 92
3. Beastall C and Shepherd CR. Trade In "Captive Bred" Echidnas. TRAFFIC Bulletin. 2013; Volume 25: page 16 – page 17

398. THE CADNAP PROFICIENCY TEST – A QUALITY CONTROL INSTRUMENT FOR PERFORMANCE MONITORING THE FORENSIC ANALYSIS OF CANINE DNA

Berger B.[1], Berger C.[1], Hecht W.[2], Hellmann A.[3], Rohleder U.[3], Schleenbecker U.[3], Morf N.[4], Parson W.[1,5]

[1] Institute of Legal Medicine, Medical University of Innsbruck, Austria
[2] Institute of Veterinary Pathology, Justus-Liebig-University Giessen, Germany
[3] Bundeskriminalamt, Kriminaltechnisches Institut, Wiesbaden, Germany
[4] Institute of Legal Medicine, University of Zürich, Switzerland

[5] Forensic Science Program, The Pennsylvania State University, PA, USA

burkhard.berger@i-med.ac.at

Identity testing of domestic dogs (Canis familiaris) has repeatedly demonstrated its capability to add relevant information to forensic cases and plays a leading role in setting quality standards for non-human DNA typing. However, canine DNA analysis is miles away from being widespread practice. That in turn means that the laboratories concerned operate isolated from others and have limited opportunities to compare their competence. Errors, bias or significant methodically differences might be serious consequences. Therefore, appropriate procedures are necessary to counteract these potential risks, if possible in advance. In order to meet this issue the CaDNAP group [gerichtsmedizin.at/cadnap.html] has successfully performed validation studies including interlaboratory comparisons (IC) for specified canine STRs [Berger et al. 2014; FSI Gen 8, 90-100]. According to ISO/IEC 17025 a laboratory shall have quality control procedures in place such as the participation in proficiency tests (PT). Proficiency testing is defined as the determination of a laboratory's testing performance against pre-established criteria by means of IC providing an independent appraisal to reference values [ILAC-P9:06/2014]. In the 2014-CaDNAP-meeting the group decided to institutionalize a biannual PT and therefore to develop a framework of rules and criteria for forensic canine DNA competence testing encompassing STRs and the mtDNA control region. The general policy of the PT closely follows the model provided by GEDNAP, which sets the benchmark for human DNA proficiency testing for decades. The general outline and a first evaluation of results of the 2015-CaDNAP-PT are presented supplemented by a review of the methods applied by the participating laboratories.

399. FORENSIC IDENTIFICATION OF SPECIES BY THE SEQUENCE ANALYSIS OF CYTOCHROME B

Farkas Z. S., Barany G., Heinrich A., Szabolcsi Z., Pamjav H., Volgyi A.

Institute of Forensic Medicine, Network of Forensic Science Institutes, Budapest, Hungary

baranyg@iszki.hu

The identification of species from biological evidence collected from crime scene might be asked for during police investigations. DNA sequence of cytochrome b gene on the mitochondrial genome can be used for species identification in the field of forensic genetics.

The aim of this study was to establish the conditions for species identification of several animal and human species based on the available GenBank data in 2015, publications and laboratory work design.

First, DNA sequences from GenBank were collected (March 2015) to construct a database of several species based on approximately 200 complete cytochrome b sequences of each animal. Consensus sequences for the animals and the human were constructed and the target region by which species identification is going to be performed was determined. Phylogenetic tree was constructed by the maximum-likelihood method to characterize the genetic distance of the species based on the complete and the target region. Degenerated universal primer pair was designed to the target region and the PCR conditions were set.

The verification of the tests was performed on collected samples from 6 species of 15 livestock animals from the Budapest Zoo and Veterinary L. Farkas (2 *Equus caballus*, 2 *Equus asinus*, 2 *Bos taurus*, 2 *Capra hircus*, 6 *Ovis aries* and 1 *Sus scrofa*). The extracted DNA was amplified, sequenced and analysed; so far no deterioration from the consensus sequences were detected, the expert's testimony would be unambiguous, partly because of the relatively large genetic distances among species. Validation of the workflow is still in progress.

400. MITOCHONDRIAL DNA CONTROL REGION VARIABILITY IN HUNGARIAN CANINE BREEDS: A FORENSIC APPLICATION

Stégmár B.[1], Egyed B.[1], Zenke P.[2,4], Pádár Z.[3,4], Vellai T.[1]

[1] *Department of Genetics, Eötvös Loránd University, Budapest, Hungary*
[2] *Faculty of Veterinary Science, Szent István University, Budapest, Hungary*

[3] Forensic Research and Criminology Centre, Széchenyi University, Győr, Hungary
[4] GenExpert Ltd., Szentendre, Hungary

stegmarb@gmail.com

Dog hairs are often found at crime scenes as evidence material, and in many cases investigation of animal traces can solve a case. Shed hairs recovered as evidence are usually telogen phase and contain minimal amount or no nuclear DNA, and therefore mtDNA can be investigated in such cases because of its high copy number in hairs. Many population studies exist of dogs' mtDNA, but none was conducted in Hungary before. In a recent research, we sequenced the entire mtDNA control region of 29 dogs from Hungarian breeds. The sample pool was selected to represent the Hungarian dog population, and therefore typical Hungarian canine breeds (like Puli, Hungarian Long- and Wire-haired Pointing Dogs, Hungarian Greyhound, etc.) were covered by the study. Apart from dogs, two wolves and one wolf-dog hybrid were also analyzed. The main goals of this study were: (1) to create an mtDNA control region sequence database of the Hungarian dog population; (2) to develop a robust method for an overlapping direct PCR sequencing of canine mtDNA to identify degraded forensic samples.
To determine dog haplotypes, each sequence was aligned to the dog reference sequence [1]. Altogether 15 dog and 2 wolf haplotypes were observed and it was found that each was previously described. The random match probability calculated only for the dog sequences was considerably high (RMP=0.12). We compared our dataset to other studies from Belgium [2,3] and from the US [4,5] applying AMOVA, and high similarities among the populations were determined (Φst=0.0086).

1. Kim KS, Lee SE, Jeong HW, Ha JH (1998) The complete nucleotide sequence of the domestic dog (Canis familiaris) mitochondrial genome. Molecular Phylogenetics and Evolution 10: 210–220.
2. Bekaert B, Larmuseau MH, Vanhove MP, Opdekamp A, Decorte R (2012) Automated DNA extraction of single dog hairs without roots for mitochondrial DNA analysis. Forensic Science International Genetics 6: 277–281.
3. Desmyter S, Gijsbers L (2012) Belgian canine population and purebred study for forensics by improved mitochondrial DNA sequencing. Forensic Science International Genetics 6: 113–120.
4. Gundry RL, Allard MW, Moretti TR, Honeycutt RL, Wilson MR, Monson KL, Foran DR (2007) Mitochondrial DNA analysis of the domestic dog: control region variation with- in and among breeds. Journal of Forensic Sciences 52: 562–572.
5. Webb KM, Allard MW (2009) Identification of forensically informative SNPs in the domestic dog mitochondrial control region. Journal of Forensic Sciences 54: 289–304

401. INCREASING RELEVANCE OF NON-HUMAN GENETICS IN HUNGARIAN FORENSIC PRACTICE

Pádár Z.[1], Zenke P.[2], Egyed B.[3], Kovács G.[4]

[1] Research Centre for Forensic Sciences and Criminology, University Széchenyi István, Győr, Hungary, GenExpert Ltd. Hungary
[2] Dept. of Animal Breeding and Genetics, University Szent István, Faculty of Vet. Sci., Budapest, Hungary, GenExpert Ltd. Hungary
[3] Dept. of Genetics, University Eötvös Loránd, Budapest, Hungary, GenExpert Ltd. Hungary
[4] Research Centre for Forensic Sciences and Criminology, University Széchenyi István, Győr, Hungary

In step with the evolution of the molecular genetics, the application of non-human biological traces is an intensively researched field in forensic sciences (1). Recently it has been widely acknowledged that this extended possibility – with associated abilities and pitfalls - for forensic DNA examination can provide innovative results in criminal and civil casework as well as in connection with associative evidence.

Depending on the specific species or types of traces, the final results can have very different criminalistics value (e. g. plants, dogs or microbiome of soil traces), but it is unquestionable that non-human DNA has an increasing role in

forensic biological evidence (2, 3, 4).

However, in contrast to the investigation of human DNA-traces, there currently exist no restrictions concerning DNA loci and privacy of genetic information, and a wide spectrum of genetic information and technologies may be applied. The field is developing with animal rights, international (e.g. CITES) conventions, national regulations and professional recommendations (5, 6, 7).

The Hungarian forensic practice on animal DNA examination has been developing since 2000 (8). The range of cases is sufficiently broad, and includes fatal accidents and animal attacks (Case #1) as well as illegal trade cases (Case #2). In case of degraded animal hair samples we used two sets of *Canine* STR markers with shortened amplicons (Case #1), and species specific primers (*Ursus sp.*) for mitochondrial DNA analysis of mixed samples (Case #2), respectively. The results supported the evaluation of individualization (Case #1), confirming the species identification (Case #2).

1. Amorim, A. Introduction to the Special Issue on Forensic Genetics: Non-Human DNA. The Open Forensic Science Journal, (2010) 3, 6-8.
2. Koopmann, W., et al. Botanical DNA evidence in criminal cases: Knotgrass (Polygonum aviculare L.) as a model species. Forensic Sci Int Genet (2012) 6: 366-374.
3. Berger, B., et al. Validation of two canine STR multiplex-assays following the ISFG recommendations for non-human DNA analysis. (2014) Forensic Sci Int Genet 8(1):90-100
4. Dawson, L. Research Into the Use of Soil Microbial Characterisation in Forensic Case Work: MiSAFE, an EU Funded Collaborative Research Project. 3rd ENFSI APST WG Meeting, Rome, Italy, (2014) http://www.socgeol.it/files/download/Rendiconti/pdfvol30_suppl_1/04_dawson.pdf
5. Budowle, B., et al. Recommendations for animal DNA forensic and identity testing. Int J Legal med (2005) 119: 295-302.
6. Linacre, A., et al. ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. Forensic Sci Int Genet (2011) 5: 501-505.
7. ENFSI Animal, Plant and Soil traces Working Group. http://www.enfsi.eu/about-enfsi/structure/working-groups/animal-plant-and-soil-traces
8. Padar, Z., et al. Canine microsatellite polymorphism as a resolution of an illegal animal death case in a Hungarian Zoological Garden. Int J Legal Med. (2001) 115: 79-81.

402. DEVELOPMENT OF A MULTIPLEX SYSTEM FOR IDENTIFYING INDIVIDUALS OF ANDEAN CONDOR (VULTUR GRYPHUS)

Paz-y-Miño C.[1], Navarrete J.[1,2], Sánchez M. E.[1], Gaviria A.[3], Leone P. E.[1], Cabrera-Andrade A.[1], López-Cortés A.[1], Burgos Figueroa G.[1]

[1] *Instituto de Investigaciones Biomédicas, Universidad de las Américas, Quito, Ecuador*
[2] *Escuela de Medicina Veterinaria y Zootecnia, Universidad de las Américas, Quito, Ecuador*
[3] *Laboratorio de Genética Molecular, Cruz Vital - Cruz Roja Ecuatoriana, Quito – Ecuador*

anibalgaviria@hotmail.com

The Andean condor (Catharthidae) is one of the largest flying birds in the world, which wide geographical distribution goes from northern South America to southern Chile. Condor is considered a patriotic symbol in five Latin-American countries; despite this, it is endangered throughout entire region. It has been reported a relatively low genetic variability in mitochondrial DNA[1], probably due to increased inbreeding in instance of size reduction of local populations. Less than fifty Andean condors exists in Ecuador, therefore activities that tend to estimate the genetic variability are considered extremely urgent if is decided to undertake a project to reintroduce this species. Consequently, those data could suggest breeding pairs each having the lowest level of consanguinity possible, based on animals held in captivity. This work reports the first STR genetic identification system for the Andean Condor, based in primer microsatellite sequences originally designed for the Californian Condor (*Gymnogyps*

californianus)[2]. Multiplex PCR amplification protocol was standardized using fluorolabelled primers for six STRs loci (1 dinucleotide, 2 trinucleotides and 3 tetranucleotides). Amplified DNA was separated by capillary electrophoresis, for allele sizing panels and bins were designed based on Genemapper V3.2 technical recommendations.

1. Hendrickson SL, Bleiweiss R, Matheus, JC, et al: Low genetic variability in the geographically widespread Andean condor. The Condor, 2003; 10(1): 1–12.
2. Romanov M, Tuttle E, Houck M, et al: The value of avian genomics to the conservation of wildlife. BMC Genomics, Proceedings of the Avian Genomics Conference and Gene Ontology Annotation Workshop, 2009. 10 (Suppl 2):S10.

403. BARCODE ANALYSIS USING MINI-AMPLICONS STRATEGY FOR MUSEUM SAMPLES OF THE NEOTROPICAL PRIMATE CALLITHRIX SPP

Loiola S.[1], Carvalho R. S.[1,2], Bergallo H. G.[3], Weksler M.[4], Carvalho E. F.[1], Silva D. A.[1]

[1] Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Brazil
[2] Programa de Pós-graduação em Ecologia e Evolução, Universidade do Estado do Rio de Janeiro, Brazil
[3] Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Brazil
[4] Laboratório de Ecoepidemiologia de Doença de Chagas, Fundação Oswaldo Cruz, Instituto Oswaldo Cruz , Rio de Janeiro, Brazil

dayse.a.silva@gmail.com

Mitochondrial DNA has become an important tool in the forensic field for analyzing ancient and degraded samples that contain insufficient amounts of nuclear genomic DNA. Among studies targeting non-human species identification, one of the most frequently used fragments is the Cytochrome C Oxidase subunit I (COI), in particular a 'barcode' region located at the 5′ end of the gene containing approximately 650 base pairs (bp) flanked by regions for which universal conserved PCR primers were designed. However, genomic DNA from forensic or museum samples are frequently degraded, hampering the analysis of DNA fragments of longer than 500 bp. The main objective of this work was to develop a set of primers to amplify five shorter overlapping COI fragments from museum specimens of the neotropical primate *Callithrix* spp. The samples were obtained from taxidermyzed skins, bones, and hair tissues of 10 specimens with a range of archival age from 70 to 10 years. PCR tests were conducted for amplification of the whole barcode COI fragment of the same specimens using 2 strategies: the universal primer pair and the five mini-aplicons primers pairs. As results, the PCR conditions were established, no amplification was observed using the universal primers for the whole COI sequence. However, using the new set of mini-amplicon primers all type of samples was amplified. We conclude that the mini-amplicon strategy for COI region typing is best suited for hardly degraded samples in forensic research, and in particular for museum samples.

1. Cordula Berger and Walther Parson: Mini-midi-mito: Adapting the amplification and sequencing strategy of mtDNA to the degradation state of crime scene samples. Forensic Science International: Genetics. 2009; 3:149-153
2. Ann Bucklin, Dirk Steinke and Leocadio Blanco-Bercial: DNA Barcoding of Marine Metazoa. Annu. Rev. Mar. Sci. 2011; 3:471–508
3. Johnson RN, Wilson-Wilde L, Linacre A. Current and future directions of DNA in wildlife forensic science. Forensic Sci Int Genet. 2014; 10:1-11

404. DEVELOPMENT OF THE GENETIC MARKERS USING THE WHOLE CHLOROPLAST GENOMES FOR DISCRIMINATING NARCOTIC POPPIES

Oh H.[1], Seo B.[1], Lee S.[1], Ahn D.-H.[2], Kim S.[3], Min G.-S.[2]

[1] Department of Forensic Science Investigation, Supreme Prosecutors' Office, Seoul, South Korea
[2] Department of Biological Sciences, Inha University, Incheon, South Korea,

[3] Division of Life Sciences, Korea Polar Research Institute, KIOST, Incheon, South Korea

water@spo.go.kr

Papaver somniferum known as the opium poppy is the only species producing morphine in taxonomic level. However it is not easy to distinguish the opium poppy from many kinds of interspecific hybrids and the dynamic morphologies in environmental conditions. Thus the correct identification of the opium poppy has been the subject in decades for the international control of narcotic drugs. In the study, we aimed to develop genetic markers discriminating the opium poppy from other *Papaver* species and/or *Papaver somniferum* varieties. So far 18 universal markers used in plants were tested for this purpose but none of them was successful. We determined the whole chloroplast genomes from three *Papaver species* (*P. sominiferum, P. orientale,* and *P. roheas*) and five *Papaver somniferum* varieties (or subspecies) (*P. somniferum* subsp. *setigerum*, *P. somniferum* subsp. *paeoniflorum*, *P. somniferum* subsp. *laciniatum*, *P. somniferum* subsp. *tasmania*, *P. somniferum* var. *giganteum*) using NGS (Illumina Hiseq 2000) technology. We found three indel (insertion or deletion) regions which are specific for P. somniferum. The sequence variation (p-distance) among five *Papaver somniferum* varieties (or subspecies) was very low, but they can be divided into three distinguishable clades from phylogenetic analysis. From theses analysis, we also found four clade-specific variable regions: two for clade1, one for clade2 and one for clade3 specific regions. These clade specific regions were verified from the test using more than 100 individuals of *Papaver somniferum* varieties (or subspecies). This means that these clade specific regions can be used to develop genetic markers for discriminating narcotic poppy (*Papaver somniferum*) varieties or subspecies.

405. GENETIC APPROACH FOR SPECIES IDENTIFICATION: FORENSIC APPLICATION OF DNA BARCODING

Verzeletti A., Cortellini V., Cisana S., Pretto S., Cerri N., De Ferrari F.

University of Brescia – Department of Medical and Surgical Specialties, Radiological Sciences and Public Health - Forensic Medicine Unit - Brescia, Italy

andrea.verzeletti@unibs.it

The ambitious idea of using a short piece of DNA for large-scale species identification (DNA barcoding) is already a powerful tool for scientists and the application of this standard technique seems promising in a range of fields including forensic genetics. The Barcode of Life Database (BOLD), continuously updated, reports the sequence of DNA (648 base pairs) for the Cytochrome Oxidase I mitochondrial gene (COI) in all living species on Earth, which can be used for their identification. Many recent studies on different animal groups demonstrate the efficacy of DNA barcoding and its potentials. This study aims to look at the current method of species identification and how the COI methodology could be applied in a forensic environment.

In particular, to test the effective application of COI analysis in forensics, we created experimentally critical conditions such as environmental and mixtures, commonly find in the forensic cases, when stain morphology is often compromised by environmental conditions.

1. Wilson-Wilde L, Norman J, Robertson J, Sarre S, Georges A: Current issues in species identification for forensic science and the validity of using the cytochrome oxidase I (COI) gene. Forensic Sci Med Path. 2010; 6:233–41.
2. Dawnay N, Ogden R, McEwing R, Carvalho GR, Thorpe RS: Validation of the barcoding gene COI for use in forensic genetic species identification. Forensic Sci Int. 2007; 173:1–6
3. Ferri G, Alù M, Corradini B, Licata M, Beduschi G: Species identification through DNA „barcodes". Genet Test Mol Biomarkers. 2009;13(3):421-6.

406. THE SHARK PANEL: AN INDEL MULTIPLEX FOR SHARK SPECIES IDENTIFICATION

Amaral C. R. L.[1], Pereira F.[2], Loiola S.[1], Silva D. A.[1], Amorim A.[3,4], Carvalho E. F.[1]

[1] *Laboratório de Diagnósticos por DNA,*

Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Universidade do Porto, Porto, Portugal
[3] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Faculdade de Ciências, Universidade do Porto, Portugal
[4] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

crlamaral@yahoo.com.br

The Elasmobranchii comprises the diverse and important group of sharks and rays. The Selachii or sharks clade includes some of the ocean's largest predatory fishes, being commercially overexploited due to unsustainable fishing activities for their meat and fins. Overfishing has resulted in significant population declines and several Selachii species are now considered under high threat and facing extinction, with about 93% of its nominal species included in the IUCN Red List. Molecular data has provided important information about these species, allowing the management of natural stocks and their decline. Population genetics, connectivity data, and their population genetics knowledge are now available and play an important role on establishing conservation policies. However, despite the ecological, commercial and conservation importance, no molecular method is available to identify sharks in a forensic analytic context. As a first step in the construction of a reliable method for shark identification, we carried out a molecular systematic analysis using 46 previously published mitochondrial 16S rRNA gene sequences obtained from the NCBI database. We found that indels in the 16S rRNA gene can be used to distinguish all the analyzed shark species, including some of the most threatened according to IUCN Red List. The regions selected in this study can be used for the construction of molecular assays for shark identification in a forensic context.

1. Pereira F, Carneiro J, Matthiesen R, van Asch B, Pinto N, Gusmão L, Amorim A. Identification of species by multiplex analysis of variable-length sequences. Nucleic Acids Research. 2010. 38 (22): e203. DOI: 10.1093/nar/gkq865.

407. IDENTIFICATION OF FRESHWATER FISHES FROM THE SOUTH AMERICAN ATLANTIC RAINFOREST: THE CASE STUDY OF THE GENUS PHALLOCEROS, USING DNA BARCODING AND SPINDEL APPROACHES

Maciel V. A.[1], Amaral C. R. L.[1], Pereira F.[2], Mazzoni R.[3], Silva D. A.[1], Amorim A.[4,5], Carvalho E. F.[1]

[1] Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[2] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Universidade do Porto, Porto, Portugal
[3] Laboratório de Ecologia de Peixes, Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil
[4] Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Faculdade de Ciências, Universidade do Porto, Porto, Portugal
[5] Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

crlamaral@yahoo.com.br

The Atlantic Rainforest is one of the global conservation hotspots, representing a threatened area with high levels of endemism and species richness. The fishes from this area are one of the most exploited vertebrate groups by the international aquarium trade, an industry with an annual volume of about U$15-25 billions. The native South American genus *Phalloceros* includes species commonly known as "guppies", being found not only in South America, but also New Zeeland, Australia and Malawi, where they have been secondarily introduced with several ecological effects. We used SNPs (DNA Barcoding) and indels (SPInDel) in mitochondrial DNA (mtDNA) to investigate the molecular diversity of the species *Phalloceros anisophallos* from the coastal Atlantic drainages of Rio de Janeiro state, Brazil. Our aim is to develop a panel of mitochondrial markers to be used on the identification of guppy fish species. The molecular analyses were carried out using novel (n = 28) sequences from the mitochondrial COI gene (for DNA Barcoding) and 16S rDNA gene (for

SPInDel). We were able to determine the molecular variability of the distinct populations of *P. anisophallos*, which showed distinct patterns of shared polimorphisms. We were also able to identify target regions rich in indels to be used in a multiplex PCR approach for species identification. This study demonstrates that the combination of SNPs and indels can be informative in the species/population identification in a rich and highly endemic biome such as the Atlantic Rainforest.

1. Hebert, P.D.N., Ratnasinghan, S., Ward, J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B. 270: S96-S99. doi: 10.1098/rsbl.2003.0025.
2. Pereira F, Carneiro J, Matthiesen R, van Asch B, Pinto N, Gusmão L, Amorim A. Identification of species by multiplex analysis of variable-length sequences. Nucleic Acids Research. 2010. 38 (22): e203. DOI: 10.1093/nar/gkq865

408. BIG GAME SPECIES IDENTIFICATION BY HIGH-RESOLUTION MELTING

Lopez-Oceja A.[1], Gamarra D.[1], Jimenez S.[2], de Pancorbo M. M.[1]

[1] *BIOMICs Research Group, University of the Basque Country (UPV/EHU), Vitoria, Spain*
[2] *Dpt. Pathology and Surgery (Legal and Forensic Medicine), University of Miguel Hernández, Elche, Spain*

susana.jimenez@umh.es

Species identification by DNA barcoding like cytochrome c oxidase I and cytochrome B (CytB) genes has become a very useful tool in molecular biology in recent years. But the degradation level shown in some forensic samples reduces the usefulness of these barcode, thus the use of mini barcodes which sizes are below 200bp is recommended to achieve optimal amplifications. Conventional methodology consists in DNA amplification by using universal primers and analysis by sequencing of the amplicons. This is a complex and time consuming procedure.

The present study is focused on the identification of five cinegetic species: deer (*Cervus elaphus*), roe buck (*Capreolus capreolus*), wild boar (*Sus scrofa*), bear (*Ursus arctos*) and wolf (*Canis lupus*), all of them living in the north of Iberian Peninsula. A pair of universal primers that amplify a CytB mini barcode was used. The analysis of amplicons was performed by High-Resolution Melting (HRM) and the obtained results have shown different clusters for each species, making posible a correct identification of the five big game species here analyzed. Dog (*Canis familiaris*) and pig (*Sus scrofa domesticus*) samples were added to observe if they might mislead the nearby wild species identification such as wolf and wild boar, but even so close species have unambiguous clusters and do not interfere with the identification of their corresponding wild species.

In conclusion, HRM based on Cytb mini barcode is a precise, rapid and cost-effective methodology for the big game species.

409. EFFICACY OF 12 STRS KIT IN CATTLE GENOTYPING FOR FORENSIC PURPOSES

Gamarra D.[1], Lopez-Oceja A.[1], Jimenez S.[2], de Pancorbo M. M.[1]

[1] *BIOMICs Research Group, University of the Basque Country (UPV/EHU), Vitoria, Spain*
[2] *Dpt. Pathology and Surgery (Legal and Forensic Medicine), University of Miguel Hernández, Elche, Spain*

susana.jimenez@umh.es

Cattle (Bos taurus) is an important meat food source of humans. STR loci have been extensively used for parentage testing in high demand of animal breeding industry but have also shown useful for forensic purposes to carry out the identification of stolen animals or involved ones in traffic accidents. Far from being an isolated case, in 2013, Spanish yearbook of traffic accident showed 577 accidents for animal collision and police noticed numerous thefts in livestocks so DNA typing is necessary to identify animals and their owners. International Panel of Microsatellite for Cattle Parentage testing (ISAG panel) established recommendations including nine microsatellite and later three

additional markers as candidate loci in cattle parentage analysis (ISAG 2008).

This study, based on a data set of 431 animals, has examined the efficacy of Finnzymes Bovine Genotype™ panel 1.2 with 12 bovine STRs (BM1818, BM1824, BM2113, ETH10, ETH225, ETH3, INRA023, SPS115, TGLA122, TGLA126, TGLA227 and TGLA53) for forensic investigations. Genotypes were used to calculate marker statistics (EH, OH, power of exclusion, matching probability, power of discrimination and minimum allele frequencies) and check power of loci as a minimum standard for identity and kinship analysis.

410. SPECIES IDENTIFICATION THROUGH PYROSEQUENCING 12S RRNA GENE

Hu Z.[1,2], Wang Z.[1], Zhang S.[1], Bian Y.[1], Li C.[1]

[1] Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science, Ministry of Justice, P.R. China, Shanghai, China
[2] Department of Forensic Medicine, Medical College of Soochow University, Suzhou, China

fly_huzhen@163.com

The identification of the biological origin of a specimen is necessary in some forensic casework. Mitochondrial DNA (mtDNA) has become a useful tool in forensic identification because of the high number of copies and the lack of recombination. In this study, we amplified and sequenced a 100-bp fragment of the mtDNA 12S ribosomal RNA (12S rRNA) gene from 18 different species including human, monkey, chicken, duck, cattle, sheep, dog, SD rat, mouse, goose, pig, horse, cat, rabbit, donkey, pigeon, deer and snack. The amplified products were sequenced for specimen identification by pyrosequencing technique. The results showed that specimen origin could be accurately determined by only 12 bases. Furthermore, the origins of six unknown samples were successfully discriminated. Through pyrosequencing 12S rRNA gene, we offer a new method for discrimination of 18 species.

411. APPLICATION OF MTSNP MARKER FOR GENETIC IDENTIFICATION OF FORENSICALLY IMPORTANT SARCOPHAGID FLIES (DIPTERA: SARCOPHAGIDAE) IN CHINA

Zhang C.[1], Fu X.[1], Yang X.[1], Liu J.[1], Guo Y.[1]

[1] Department of Forensic Science, Central South University, Changsha, China

gdy82@126.com

Forensic entomology is very important to estimate the postmortem interval (PMI), especially in the investigation of corruption bodies, and then the identification of a necrophagous insect specimen is the earliest step. However, the identification of species of the forensically important sarcophagid flies (Diptera: Sarcophagidae) is very difficult and requires strong taxonomic expertise. More and more methods according to the mitochondrial DNA (MtDNA) and nuclear fragments are raised, but the current methods of molecular identification have some defects, such as insufficient discrimination power, high cost, time-consuming and so on. Now, we detected the whole sequences of MtDNA from many different species of the flesh flies, and added GenBank data to our database to yield a total dataset for selecting 8 allelic SNP markers to establish the SNP-based genotyping system. Using this system by PSQ, we can identify the species of forensically common Sarcophgid flies with high discrimination power at a low cost in the short time. The 8 SNPs marker system shows a new idea for genetic identification of forensic insects.

Theme 18: Quality Control

412. CERTIFICATION OF A DNA QUANTITATION STANDARD USING DIGITAL PCR

Romsos E. L., Kline M. C., Duewer D. L., Vallone P. M.

National Institute of Standards and Technology, Gaithersburg, MD, U.S.

erica.romsos@nist.gov

The current version of SRM 2372 was designed for use in the value assignment of human genomic DNA forensic quantitation materials and was originally certified for spectrophotometric absorbance of 260 nm [1]. However in 2012, five years after production, the measured absorbance in all materials had increased (by approximately 9 % to 14 %) to the point that the certified values were no longer within the stated error. Investigation revealed that the increase resulted from deshielding due to tertiary structure changes [2,3]. Due to this phenomenon and the projected depletion of SRM 2372, the next iteration of SRM 2372 will be certified for copy/target number using digital PCR (dPCR). Digital PCR depends on partitioning PCR reactions either a microfluidic platform (chamber digital PCR, cdPCR) or in a droplet emulsion (droplet digital PCR, ddPCR). This partitioning allows the estimation of the number of accessible amplifiable targets without an external calibrant. The use of digital PCR enables direct determination of accessible amplifiable genomic targets. Initial experiments suggest that the nine PCR assays agree within 5 % to 10 % across all platforms. This presentation will discuss the importance of assessing the variability at the genome level in terms of copy number variants by utilizing nine single copy assays evaluated and optimized for amplifiable accessibility across three platforms (qPCR, cdPCR, ddPCR). Comparison between these platforms for designing and optimizing an assay will be discussed.

References:

1. Kline, M.C., Duewer, D.L., Travis, J.C., Smith, M.V., Redman, J.W., Vallone, P.M., Decker, A.E., Butler, J.M. (2009) Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard. Anal. Bioanal. Chem. 394: 1183-1192.
2. Vallone, P.M., Butts, E.L.R., Duewer, D.L., Kline, M.C. (2013) Recertification of the NIST Standard Reference Material 2372®, human DNA quantitation standard. Forensic Sci. Int. Genet.: Suppl. Ser. 4(1): e256-e257.
3. Tinoco I Jr. Hypochromism in Polynucleotides. (1960) J Am Chem Soc 82(18):4785–4790.

413. ACCREDITATION OF THE GHEP-ISFG PROFICIENCY TEST: ONE STEP FORWARD TO QUALITY ASSURANCE AND IMPROVEMENT OF THIS FORENSIC INTERCOMPARISON EXERCISE

Fernández K., Gómez J., García-Hirschfeld J., Cubillo E., Sánchez de la Torre C., Vallejo G.

National Institute of Toxicology and Forensic Sciences (INTCF), Ministry of Justice, Madrid, Spain

coro.fernandez@justicia.es

Performing proficiency tests is an essential component of a laboratory's quality assurance program, enabling laboratories to monitor and demonstrate the quality of their analytical results. The ongoing confidence in proficiency tests requires a fully assurance in their testing schemes. ISO/IEC 17043 is the fundamental standard which ensures quality of proficiency testing providers regarding technical (personnel, design of proficiency testing schemes, preparation of items, assigned values, data analysis & evaluation, and reports) and management (management system, internal audits, control of records, subcontracting services and reviews) requirements (1). Since 1992, it has being organized annually in Spain, a Forensic Intercomparison Exercise coordinated by the Madrid Department of the INTCF and organized by the Spanish and Portuguese Speaking Working Group of the International

Society for Forensic Genetics (GHEP-ISFG). The need to improve and assure the quality of our services as well as to demonstrate our competence, led us to accredit this Exercise. In this work, we present the step-wise process we began in 2011 focused on upgrading the Basic level of the Intercomparison Exercise towards its accreditation, which was successfully achieved in December 2014. All adjustments made are described. From the early structural changes, since the Exercise had to be divided in two levels, passing through the definition of the scope, to the technical improvements made (documentation of procedures, of planning & design, homogeneity and contamination studies, clear establishment of assigned values, fluent communication with participants..) and management policy implementation (well-established procedures for complaints and appeals, internal audits programs, management reviews..) among others.

1. ISO/IEC 17043:2010. Conformity assessment. General requirements for proficiency testing.

414. DNA CONTAMINATIONS VERSUS COMPLEX DNA TRACES

Poehlmann T.[1,2], Jäger S.[2]

Tobias.Poehlmann@uni-jena.de

Contamination analysis is a major hurdle in forensic science. Contaminations can arise from various sources including lab staff, materials, ladders and other samples. Whereas contaminations from material and ladders appear to be manageable, the prevention staff-contaminations requires much attention regarding regulatory principles. Contaminations arising from other samples – even when they appear very rarely – are hard to detect. LIMS-software can support to manage both, law requirements regarding staff DNA-databases and it has the biostatistical potential to detect contaminations within trances. Intelligent staff databases (based on user login activity) optimize the risk of contaminations and automatically clear databases from inactive staff members. Biostatistical tools can help to detect contaminations within a sample and can be used to interpret data from mixed traces. Especially when samples display bad DNA quality, contain multiple DNA sources or even various species, biostatistical tools may rescue analyses. Our vision is to further develop established LIMS-software from a tool to record lab activity to actively support forensic science.

1. Dr. Tobias Poehlmann, Friedrich-Schiller-University of Jena, Germany
2. Stefan Jäger, Optimal Systems GmbH Jena, Germany

415. EDNA - AN EXPERT SYSTEM TO SPEED UP FORENSIC DNA CASEWORK

Neuhaus-Steinmetz U.[1], Haldemann B.[1], Dornseifer S.[1]

[1] Kriminaltechnik, der Polizeipräsident in Berlin, Berlin, Germany

Dr.Ulrich.Neuhaus-Steinmetz@polizei.berlin.de

Severe backlogs in forensic DNA casework are recognized in many countries. To fight these backlogs an expert system for automated comparison of DNA profiles (matching) is currently developed. It is meant to support the forensic DNA expert in his probably most tedious and error-prone task, i.e. comparing stains and reference profiles allele by allele. Intelligent visual support of profile evaluation and match interpretation by an intuitive graphical user interface (GUI) as well as automated report generation will facilitate the work of the expert and speed up forensic DNA analysis.

The currently developed expert system is anticipated to evolve into a powerful analysis tool for DNA casework providing the following features:

- Matching of reference profiles with stains
- Adequate consideration of replicates
- Quality classification
- Assessment of the minimal number of contributors
- Evaluation of usability for national DB entry (DAD)

- Automated report generation, including tables and text blocks
- Fast and easy data import and export routines
  - Standard input formats (e.g. GeneMapper®)
  - Customizable output formats
  - Interfaces to local LIMS and other external software (e.g. statistic tools)

As the experience with a precursor macro-based version revealed casework can be enhanced at least two-fold by the automation of DNA profile matching and report writing.

This is the first live demonstration of a beta version of the software. eDNA will be freely available for registered users in the first half of the year 2016.

416. THE VIRTUAL DNA SCIENTIST: HOW TWO CRIME LABS USED AUTOMATION TO INCREASE DNA SCIENTIST SAMPLE OUTPUT BY MORE THAN 40%

Stacy T.[1], MacIntosh H.[2], Hoey B.[3], Squibb M.[4]

[1] STACS DNA, Fairfax, VA, USA
[2] STACS DNA, Ottawa, ON, Canada
[3] Missouri State Highway Patrol, Jefferson City, MO, USA
[4] Miami Valley Regional Crime Lab, Dayton, OH, USA

heather.macintosh@stacsdna.com

DNA scientists are the greatest asset of any DNA laboratory. They provide labour and expertise to perform testing and complete cases, accomplishing the fundamental work of the lab. They also represent a large liability, as salaries, benefits, vacations and training add up to big recurring costs. As crime labs grapple with case backlogs, a common approach is to hire additional DNA scientists. The hiring approach creates two new challenges. First, it takes up to two years to hire and train a scientist to process cases, meaning a considerable delay and huge investment before he or she can process a single sample. Second, once the backlog is eliminated, there may be too many scientists on the payroll for the typical number of DNA cases it receives. Bar code readers, instrumentation and software have enabled DNA scientists to complete far more cases than was possible using manual methods. Investing in technology could enable current staff to substantially increase productivity while spending less money – without any new hires. This poster examines how two laboratories – a small regional laboratory and a larger multi-site state laboratory – used technology to increase throughput by at least 40% without hiring. In essence, by embracing technology, they have created Virtual DNA Scientists who work 24/7 with no salary or benefits. The case studies illustrate how these labs eliminated their DNA backlog without any hiring. Management can determine the maximum case output per current employee and the right staffing/ technology mix to best address the lab's DNA case load.

417. RESULTS OF THE 2015 RELATIONSHIP TESTING WORKSHOP OF THE ENGLISH SPEAKING WORKING GROUP

Friis S. L.[1], Hallenberg C.[1], Simonsen B. T.[1], Morling N.[1]

[1] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

susanne.friis@sund.ku.dk

The English Speaking Working Group of the International Society for Forensic Genetics annually offers an exercise involving genetic analysis in a relationship case with the objective to compare the results obtained in the participating laboratories.

In 2015, the exercise included paternity testing of blood samples from a mother, two children and an alleged father. The samples were distributed to 63 laboratories together with a questionnaire and a paper challenge. The paper challenge contained typing results of a child and an alleged grandfather. Database information about the allele distributions in the tested systems was provided, and the laboratories were asked to submit results of the biostatistical

calculations and the formulas used. The paper challenge included autosomal and Y-chromosomal STR data.

The presentation of the 2015 Relationship Testing Workshop will include concordance/discordance in typing results, collation of systems and kits used by the laboratories and an evaluation of methods and strategies applied for DNA-typing. We present a comparison of the requirements given by the laboratories to issue a paternity case with an excluded man and with a non-excluded man, respectively. Furthermore, the results of the biostatistical calculations of the paper challenge will be presented and discussed.

418. COLOMBIAN RESULTS OF THE INTERLABORATORY QUALITY CONTROL EXERCISE 2013-2014

Builes J. J.[1,2] , Manrique A.[1,3], Aguirre D. P.[1], Mendoza L.[1,4], Bravo M. L. J.[1], Carmona V.[1,2], Afanador C. H.[5], Salgar M.[1,2], Lopez M.[1], Gusmão L.[6,7]

[1] GENES SAS Laboratory, Medellín, Colombia
[2] Institute of Biology, University of Antioquia, Medellín, Colombia
[3] School of Medicine, University of Campinas, São Paulo, Brazil
[4] Universidad Pontificia Bolivariana, Medellín, Colombia
[5] School of Medicine, University of Antioquia, Medellín, Colombia
[6] IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal
[7] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil.

jjbuilesg@une.net.co; genforense@genesltda.com

Currently, all agencies of conformity assessment testing laboratory favor the participation in proficiency testing as a mechanism for quality assurance. In Colombia, the Genes Laboratory has been designated, since 2008, to perform the design and implementation of proficiency testing for all the interested laboratories. In this report, the results of Colombian exercises Inter-laboratory Quality Control for the years 2013 and 2014 are presented. In both years the exercise consisted in one practical component, one theoretical mandatory component and a theoretical optional component. The participants were 23 and 20 laboratories in 2013 and 2014, respectively, representing seven different countries of Latin America and the Caribbean. For the practical component each participant laboratory receive; (1) samples of blood, saliva and/or semen stains, in this part they should report the routine own laboratory markers for each sample, (2) three optional theoretical cases of varying complexity, (3) a simple theoretical approach consistent of paternity case (father, mother and son) (2013) and a complex paternity case with a deceased father (2014). In the last two components of the exercise they had to submit only the calculations. In both years, for the practical component the consensus of 70 STR markers, distributed between autosomal and linked to the sex chromosomes, was achieved with an error rate of 1.75% and 3.07% for 2013 and 2014, respectively. On the other hand, for mandatory theoretical exercise, error rates of 21.74% (2013) and 10.63% (2014) were detected. This inter-laboratory exercise has become an important mechanism for quality assurance in the region.

419. A SURVEY OF ENVIRONMENTAL DNA IN SOUTH AUSTRALIAN POLICE LABORATORIES

Henry J.[1], McGowan P.[2], Brown C.[2]

[1] Forensic Science SA, Adelaide, Australia
[2] South Australian Police, Adelaide, Australia

julianne.henry@sa.gov.au

Crime scene exhibits are sometimes examined and sampled in South Australian Police (SAPOL) laboratories prior to submission to Forensic Science SA (FSSA) for DNA profiling. There are 18 such laboratories, including metropolitan and regional police stations, Fingerprint Bureau and Forensic Response Section (FRS).

Forensic Science SA has employed an environmental DNA survey program since 2008 as part of its Quality Assurance system. This program aims to measure the background levels of DNA in areas where forensic evidence is

collected, examined or processed to ensure that cleaning regimes are effective and DNA contamination is minimised.

This is the first survey of environmental DNA in SAPOL laboratories by FSSA and our results show that background contamination is present and poses a potential risk to forensic exhibits. The results of this study will be presented along with potential changes to laboratory practice that SAPOL could adopt to minimise the potential for DNA contamination.

420. ACCREDITATION OF FORENSIC LABORATORIES AS A PART OF THE „EUROPEAN FORENSIC SCIENCE 2020" CONCEPT IN V4 COUNTRIES

Pádár Z., Nogel M., Kovács G.

Research Centre for Forensic Sciences and Criminology, University Széchenyi István, Győr, Hungary

Globalisation and cross-border crime has led EU Member States to improve methods for fighting crime on an international level as well as to increase the mutual recognition of each state's evidence collection and analysis. In the interest of the regulated and scheduled implementation of the harmonization process, the Council of the EU adopted conclusions on the vision for European Forensic Science 2020 including the creation of a European Forensic Science Area and the development of forensic science infrastructure in Europe (EFSA 2020), in order to ensure the equivalence of professional forensic examinations. The collection, processing, use and delivery of forensic data will be based on equivalent minimum forensic science standards, and forensic service providers will work on the basis of a common approach to implement these standards. This will foster a closer cooperation between them and criminal justice systems. The accreditation of forensic laboratories is a cornerstone of the above mentioned objectives (1, 2).

In order to ensure the integrity of DNA profiles the Council issued decision 2008/616/JHA (3) on the application of the EN ISO/IEC 17025 (4) standard regarding the operation of testing and calibration laboratories. According to Council Framework Decision 2009/905/JHA (5) on the accreditation of judicial expert laboratories, the laboratories have to be accredited by 30th November 2015.

We investigated the regulations, decisions and rules – including national and international measures – of the given area.

1. House of Commons Science and Technology Committee of UK. Forensic Science, Second Report of Session 2013-14.
2. Roux, C., Crispino, F., Ribaux, O. From Forensics to Forensic Science, Current Issues in Criminal Justice, (2012) Vol.: 24 (1)
3. Council Decision 2008/616/JHA
4. SO/IEC 17025 General requirements for the competence of testing and calibration laboratories, http://www.standards.org/standards/listing/iso_17025
5. Council Framework decision 2009/9056JHA

421. EXPERIENCE IN FORENSIC GENETICS INTERLABOARTORY COLLABORATIVE QUALITY CONTROL EXERCISES IN ARGENTINA (1998-2014)

Marino M.[1], Beltramo J.[1], Colussi A.[1], Herrera M.[1], Martinez G.[1], Miozzo C.[1], Modesti N.[1], Raimondi E.[1], Sala A.[1], Corach D.[2]

[1] Argentinean Society of Forensic Genetics (SAGF) Directory Board, Argentina
[2] Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochesmistry, Universidad de Buenos Aires, Buenos Aires, Argentina

Since 1993, the Spanish and Portuguese Speaking Working Group of the ISFG (GHEP-ISFG) organized Inter-laboratory Collaborative Quality Control Exercises (IQCE) yearly. At that time only one Latin American laboratory participated. In 1997 a survey was done in Argentina inviting the 22 laboratories that offered

paternity tests: its results showed that twelve of them employed DNA based approaches, 17 consider the need to create a Scientific Society and 16 propose to adopt the IQCE taking the GHEP-ISFG as a model. In 1998 the first Latin American IQCE was organized in Argentina, participating 14 Argentinean and 9 foreign labs from six Latin American countries. Only 65% reported LR and all assays were performed manually. After the creation of the Argentinean Society of Forensic Genetics (SAGF, in 2000), a yearly schedule was maintained for the IQCE. Along the years, a clear trend towards the increasing of public labs was evident. In the last two IQCE the number of participants climbed to 30 and 64% of them belonged to public institutions (Justice and University) versus the 47% in 1998. The minimum number of Autosomal STRs analyzed was 13 and maximum 29, 25 laboratories use Y-STRs and 10 analyzed mtDNA and X-STRs. In IQCE 2014, 100% of laboratories reported LR and employed automated platforms. This work shows the evolution of IQCE in the last 16 years and reflects the great effort made by the SAGF in their organization, contributing to improve the technical proficiency and the quality of most laboratories in Argentina and the region.

422. UPDATE OF NIST SRM 2391C: PCR-BASED DNA PROFILING STANDARD WHAT IS NEW?

Steffen (Hill) C. R.[1], Gettings K. B.[1], Kline M. C.[1], Duewer D. L.[1], Vallone P. M.[1]

[1] *National Institute of Standards and Technology, Gaithersburg, MD, United States*

becky.hill@nist.gov

The NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling Standard was recently updated to contain new information relevant to the forensic community. Previously, there were certified genotypes for 24 autosomal STR markers plus Amelogenin and 17 Y-STR markers. Due to the increase in markers present in larger commercial autosomal STR and Y-STR multiplex kits recently released, there is a need to add certified types for these new markers for each component of SRM 2391c (Components A-F). The updated Certificate of Analysis has certified values for 1 additional autosomal STR marker (D6S1043) and 12 additional Y-STR markers (29 total) as well as informational values for X-STR markers (12 total) and Insertions and Deletions (Indels) (30 total). Also, the number of STR multiplex assays tested increased from 24 to 43. Sanger sequencing was performed on Components A-C, E and F (Component D is a mixture of Components A and C) to determine the STR repeat motifs and to characterize adjacent flanking regions and underlying polymorphisms (sequence, insertion-deletion, variation in complex motifs) typically not detected by fragment-based typing. The sequenced regions include the commercial or known PCR binding sites commonly implemented in fragment-based typing. The certified, reference, and information values will be presented. The sequence motifs of the single source components further characterized by Sanger and next-generation sequencing will illustrate interesting flanking region polymorphisms and motif variations.

423. PROFILE MAPPING DNA LABORATORIES OVERLOOKING THE FORENSIC FIELD AND USE OF QUALITY SYSTEMS

Rover T.[1], Borges R.[1], Silva D. A.[2], Carvalho E. F.[2]

[1] *General Coordination for Accreditation, Inmetro, Rio de Janeiro, Brazil*
[2] *DNA Diagnostic Laboratory, State University of Rio de Janeiro, Rio de Janeiro, Brazil*

elizeufc@hotmail.com

The correct technical and scientific understanding as well as the quality control protocols for the DNA analysis has been important in legal decisions in the light of technological development in the last 20 years. This study aimed to map the quality in DNA laboratories to meet the standards used by these laboratories. A survey was conducted to identify laboratories that work in forensics and quality requirements used. The results on the responses of 34.7% of laboratories (N = 72) point distribution of 44% of laboratories in the axis Rio de Janeiro and São Paulo. The paternity testing and forensic analysis represent 72% of the purposes of the analysis of DNA in the country. For the assurance of the measurement results, the survey showed that 76% use internal control

and reference materials. Regarding the participation in proficiency testing, relevant factor for the external control, 72% use such mechanism. As for the implementation of quality management systems, research shows that 20% have implanted system and 16% by implantation, with the standard ISO / IEC 17025 the reference to the forensic field. This survey concluded that DNA laboratories aimed at implementation of quality management systems to guarantee reliable and comparable measurement results nationally and internationally. In the search for these improvements and considering international guidelines, it is clear that the use of quality requirements are extremely important in order to harmonize procedures and allow to obtain reliable and comparable measurement results.

References:

1. Giovanelli, A, Garrido, RG. The criminal forensics in Brazil as legitimizing of inquisitorial police practices. Revista LEVS 2011; 7(7):5-23. Available in: http://revistas.marilia.unesp.br/revistas/index.php/levs/article/download/1672/1420. Accessed in 08 Jan 2015.
2. Malkoc, E, Neuteboom, W. The current status of forensic science laboratory accreditation in Europe. Forensic Science International 2006; 167, 121-126.
3. Godinho, NMO. DNA Bank data: one in the service of justice tool. Rev Bras de Est de Seg Púb [Internet] 2014; 7(2):20-30. Available in: http://revista.ssp.go.gov.br/index.php/rebesp/article/viewFile/193/82. Accessed in 06 Jan 2015.
4. Associação Brasileira de Normas Técnicas (ABNT). ABNT NBR ISO/IEC 17025:2005, 2005. Rio de Janeiro. ABNT
5. Silva, ACA, Gontijo, CC. Accreditation, validation and verification at forensic practices. Rev CPGLS UCG 2010. Available in: http:// www.cpgls.ucg.br. Accessed in 06 Jan 2015.

INDEX OF AUTHORS

A

B

C

D

E

F

G

H

I

J

K

L

Ł

M

N

O

P

R

S

T

U

V

W

X

Y

Z

DTP & Print:
EURO GRAPHIC
www.egn.pl

GENERAL INFORMATION

ACCOMMODATION

Below is the list with the contact information of all the Congress' Hotels.

1 Andel's Hotel Cracow****

Address: Pawia 3, 31-154 Krakow
Telephone: +48 12 299 00 97
E-mail: reservation@andelscracow.com
Website: www.vi-hotels.com/pl/andels-cracow

2 Excelsior Boutique Hotel****

Address: Pilsudskiego 23, 31-110 Krakow
Telephone: +48 12 354 30 00
E-mail: infobis@hotel-fortunabis.pl
Website: www.perfecthotels.pl

3 Florianska Guesthouse

Address: Florianska 49, 31-019 Krakow
Telephone: +48 12 421 12 25
E-mail: dguj@uj.edu.pl
Website: www.dg.uj.edu.pl/En_Gb/Florianska

4 Fortuna Hotel***

Address: Czapskich 5, 31-110 Krakow
Telephone: +48 12 422 31 43
E-mail: info@hotel-fortuna.com.pl
Website: www.hotel-fortuna.com.pl

5 Fortuna Bis Hotel***

Address: Pilsudskiego 25, 31-110 Krakow
Telephone: +48 12 430 10 25
E-mail: infobis@hotel-fortunabis.pl
Website: www.hotel-fortunabis.pl

6 Holiday Inn Krakow City Center*****

Address: Wielopole 4, 31-072 Krakow
Telephone: +48 12 619 00 51
E-mail: reservation@hik.krakow.pl
Website: www.hik.krakow.pl/?lang=en

7 Ibis Budget Krakow Stare Miasto Hotel*

Address: Pawia 11, 31-154 Krakow
Telephone: +48 12 355 29 50
E-mail: H7165@accor.com
Website: www.ibis.com/gb/hotel-7165-ibis-budget-krakow-stare-miasto/index.shtml

8 Ibis Krakow Centrum Hotel**

Address: Syrokomli 2, 30-102 Krakow
Telephone: +48 12 299 33 00
E-mail: H3710@accor.com
Website: www.ibis.com/pl/hotel-3710-ibis-krakow-centrum/index.shtml

9 Kossak Hotel****

Address: Kossaka 1, 31-106 Krakow
Telephone: +48 12 379 59 00
E-mail: kossak@hotelkossak.pl
Website: www.hotelkossak.pl

10 Logos Hotel***

Address: Szujskiego 5, 31-123 Krakow
Telephone: +48 12 631 62 77
E-mail: jacek.piskorz@hotel-logos.pl
Website: www.hotel-logos.pl

11 Maksymilian Hotel***

Address: Karmelicka 36, 31-128 Krakow
Telephone: +48 12 341 44 93
E-mail: rezerwacja@hotelmaksymilian.pl
Website: www.hotelmaksymilian.pl

12 Metropolis Design Hotel****

Address: Wygoda 8, 31-106 Krakow
Telephone: +48 12 446 90 90
E-mail: hotel@MetropolisDesignHotel.pl
Website: www.metropolisdesignhotel.pl/en/index.html

13 Novotel Krakow Centrum Hotel****

Address: Kosciuszki 5, 30-105 Krakow
Telephone: +48 12 299 29 00
E-mail: h3372@accor.com
Website: www.accorhotels.com/gb/hotel-3372-novotel-krakow-centrum/index.shtml

14 Ostoya Palace Hotel****

Address: Pilsudskiego 24, 31-109 Krakow
Telephone: +48 12 430 90 00
E-mail: hotel@ostoyapalace.pl
Website: www.ostoyapalace.pl

15 Radisson Blu*****

Address: Straszewskiego 17, 31-101 Krakow
Telephone: +48 12 618 88 88
E-mail: marta.switala@radissonblu.com
Website: www.radissonblu.com/hotel-krakow

16 Senacki Hotel****

Address: Grodzka 51, 31-001 Krakow
E-mail: senacki@hotelsenacki.pl
Website: www.hotelsenacki.pl

17 Stanisław Pigon Visiting Professors' House

Address: Garbarska 7a, 31-131 Krakow
Telephone: +48 12 422 30 08
E- mail: pigoniowka@uj.edu.pl
Website: www.dg.uj.edu.pl/en_GB/garbarska

18 Student Dormitory Nawojka

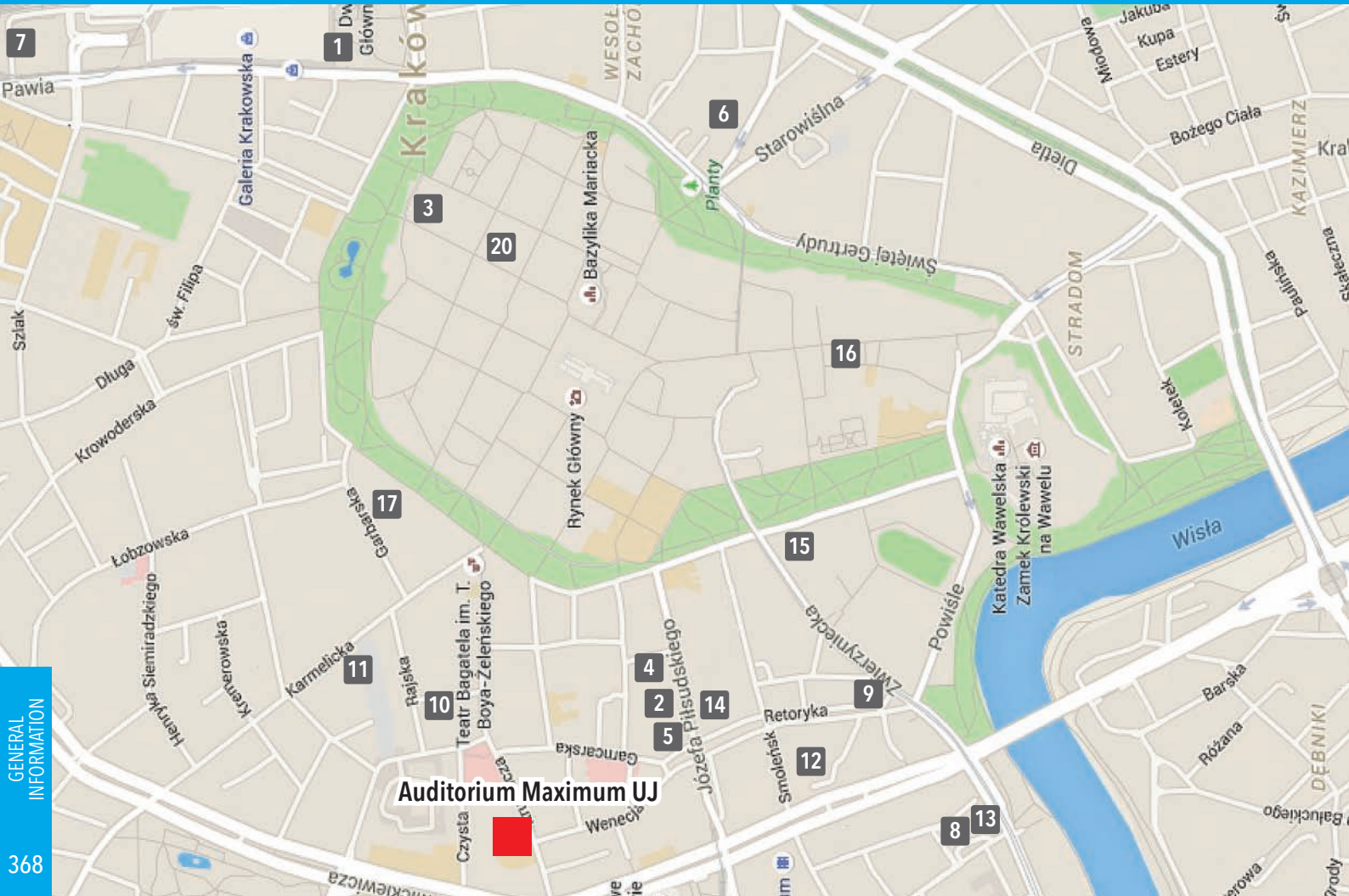
Address: Reymonta 11, 30-059 Krakow
Telephone: +48 12 378 11 00
E-mail: nawojka@hotele.studenckie.pl
Website: www.hotelestudenckie.pl/index.php?hotel-nawojka

19 Student Dormitory Zaczek

Address: 3 Maja 5, 30-063 Krakow
Telephone: +48 12 622 12 00
E-mail: zaczek@hotele.studenckie.pl
Website: www.hotelestudenckie.pl/index.php?hotel-zaczek

20 Unicus****

Address: Florianska 35/Sw. Marka 20, 31-020 Krakow
Telephone: +48 12 433 71 11
E-mail: recepcja@hotelunicus.pl
Website: www.hotelunicus.pl


Auditorium Maximum UJ
Pawia
Galeria Krakowska
Główna
Kraków
Bazylika Mariacka
Planty
Starowiślna
Bożego Ciała
Dietla
Jakuba
Kupa
Estery
Młodowa
KAZIMIERZ
Świętej Gertrudy
STRADOM
Paulińska
Kołłątaja
Katedra Wawelska
Zamek Królewski na Wawelu
Wisła
Powiśle
Rynek Główny
Św. Filipa
Długa
Krowoderska
Sziak
Łobzowska
Gabarska
Karmelicka
Siemiradzkiego
Henryka
Kremerowska
Rajska
Teatr Bagatela im. T. Boya-Żeleńskiego
Garncarska
Czysta
Wenecja
Józefa Piłsudskiego
Zwierzyniecka
Retoryka
Smoleńsk
Barska
Różana
DĘBNIKI
Balickiego
3
20
6
16
17
15
11
10
4
2
5
14
9
12
8
13
1
7

GETTING TO AND FROM KRAKOW AIRPORT

John Paul II International Airport Kraków–Balice is the second largest airport in Poland. It is located in the south of Poland, 11 km west of Kraków. Krakow Airport is served by two regular bus lines: 208 and 292 and one night line: 902 (final stop: Main Railway Station Krakow). These are agglomeration bus lines. Ticket prices varies for different types of trips and types of discounts. Tickets can be purchased from: kiosks, ticket machines at the bus stops and in the buses, the bus driver. The ticket must be validated when getting on the bus. The expected journey time is 40 minutes. More information at mpk.krakow.pl

Krakow Airport Taxi is the only official taxi service at the airport. Phone booking: +48 12 258 02 58. The charges within each of the five metropolitan area zones are flat rate regardless of: chosen route and duration of the ride, time of the day, day of the week. Initial charge: PLN 0.00

Rent-a-car offices at the airport (due to the Kraków Airport investment process, car rentals have been moved to the multi-story car park): Avis (+48 12 6393289), Budget (+48 12 2855025), Dollar (+48 12 2953439), Europcar (+48 12 2855045), Express Rent A Car (+48 12 3000300), Hertz (+48 12 2855084), Enterprise Rent A Car (+48 12 2856078), Panek (+48 509811211), SIXT (+48 12 6393216).

CLIMATE

The climate in Poland is temperate. September can be sunny and dry, with average temperatures of 15-20° C during the day and 8-12° C at night. Nevertheless, rain can occur.

TIME ZONE

Poland is located in the Central European Time Zone.

BANK FACILITIES, CURRENCY AND CREDIT CARDS

Bank hours are: Monday - Friday 8:00 - 18:00. The Polish currency is the Polish złoty (abbreviation - *zł*). Its international code is PLN. 1 złoty = 100 groszy (abbreviation - *gr*). There are the following denominations: 1, 2, 5, 10, 20, 50 gr and 1, 2, 5 *zł* (coins), and 10, 20, 50, 100 and 200 *zł* (notes). Foreign currency can be exchanged at the airports, hotel receptions, banks and in small exchange offices called "kantor". The present approximate exchange rate is available at http://www.nbp.pl/homen.aspx?f=/kursy/ratesa.html. International credit cards (VISA, Mastercard, Eurocard) are accepted in

most hotels, and in many shops and restaurants. They can be also used in most ATMs, called "bankomat" in Polish.

EMERGENCIES AND INSURANCE

The emergency phone number in Poland is 112. This will connect you to an operator who will put you through to the police, ambulance service, or fire brigade. The ISFG2015 organizing committee or its agents, will not be responsible for any medical expenses, loss or accidents incurred during the conference. Delegates are strongly advised to arrange their own personal insurance to cover medical and other expenses including accident or loss. Having EHIC card is recommended for citizens from EU countries.

EATERIES

Krakow is not only famous for its scenery and history but also for its numerous restaurants and eateries dotted around the city. All kinds of diners can be found in the historical Old Town, as well as in Kazimierz (the former Jewish quarter) and Podgorze across Wisla river. Tipping is expected for good service, the norm is to tip around 10% of the bill.

The most traditional Polish dishes are: *bigos* (seasoned stew made from sauerkraut with various meats and sausages), *pierogi* (dumplings, with various fillings: sauerkraut with mushrooms, cheese and potatoes, fruits, meat), *gołąbki* (cabbage parcels stuffed with meat or meat and rice) and *barszcz czerwony* (beetroot soup with vegetables and sour cream or served clear with dumplings).

ELECTRICITY

The mains electricity in Poland is 230 volts, 50 Hz. Socket outlets are for plugs with two round pins (plug types C and E), which are common in Europe, South America and Asia.

LANGUAGE

The official language of the Congress is English. The national language is Polish. Within the City Center, signs and information at monuments and other tourist attractions are in Polish and English.

PROGRAM AT GLANCE

Monday, August 31, 2015

09:00 – 17:00	Workshop: Basic STR interpretation (Medium Hall A)
09:00 – 18:30	Workshop: EMPOP advanced practical course (Conference Room)
09:00 – 18:30	Workshop: Next Generation Sequencing (Medium Hall B)
09:00 – 18:30	Workshop: Beyond DNA-profiling: RNA-profiling, transfer and persistence – what is it and how did it get there? (Small Hall)
11:00 – 11:30	Coffee break (-1 level)
13:00 – 14:30	Lunch Break (-1 level)
16:00 – 16:30	Coffee break (-1 level)

Tuesday, September 1, 2015

09:00 – 18:30	Workshop: The interpretation of complex DNA profiles using open-source software LRmix Studio and EuroForMix (EFM) (Conference Room)
09:00 – 13:00	Workshop: Kinship analysis (Small Hall)
14:30 – 18:30	Workshop: The new Y Chromosome Haplotype Reference Database and optimized approaches for the forensic Y-STR analysis (Small Hall)
09:00 – 13:00	Workshop: Ethical, legal and social issues in forensic genetics (Medium Hall A)
14:30 – 18:30	Workshop: Forensic DNA Phenotyping: basis, availabilities and expectations (Medium Hall A)
09:00 – 17:00	Workshop: Interpretation of complex DNA profiles using a continuous model – an introduction to STRmix™ (Medium Hall B)
11:00 – 11:30	Coffee break (-1 level)
13:00 – 14:30	Lunch Break (-1 level)
16:00 – 16:30	Coffee break (-1 level)
19:00 – 22:00	Opening Ceremony (Large Hall) ISFG Scientific Prize Lecture: Peter Gill (Large Hall) Concert: Marcin Wyrostek & Tango Corazon (Large Hall) Get-Together Party (-1 level)

Wednesday, September 2, 2015

08:45 – 09:00	Opening Welcome (Large Hall)
09:00 – 09:45	Plenary lecture: Bruce Weir (Large Hall)
09:45 – 11:00	ISFG General Session Talks (Large Hall)
11:00 – 11:30	Poster viewing (Exhibition Room, Seminar Room), Coffee break (-1 level)
11:30 – 12:15	ISFG General Session Talks (Large Hall)
12:15 – 13:00	Panel discussion: Sequencing based STR nomenclature (Large Hall)
13:00 – 14:30	Lunch Break (-1 level), ThermoFisher Symposium (Medium Hall A&B)
14:30 – 15:15	Plenary lecture: Chris Tyler-Smith (Large Hall)
15:15 – 16:00	ISFG General Session Talks (Large Hall)
16:00 – 17:00	Poster viewing (Exhibition Room, Seminar Room), Coffee break (-1 level)
17:00 – 18:00	ISFG General Session Talks (Large Hall)
18:00 – 19:00	ISFG Working Group Meetings (see page 18)
20:00 – 23:00	Gala Dinner (Gallery of the 19th Century Polish Art in Sukiennice)

Thursday, September 3, 2015

09:00 – 09:45	Plenary lecture: Manel Esteller (Large Hall)
09:45 – 10:30	ISFG General Session Talks (Large Hall)
10:30 – 11:30	Poster viewing (Exhibition Room, Seminar Room), Coffee break (-1 level), Silicon Biosystems presentation (Medium Hall A&B)
11:30 – 13:00	ISFG General Session Talks (Large Hall)
13:00 – 14:30	Lunch Break (-1 level), Promega Symposium (Medium Hall A&B)
14:30 – 15:15	Plenary lecture: Robin Williams (Large Hall)
15:15 – 16:00	ISFG General Session Talks (Large Hall)
16:00 – 17:00	Poster viewing (Exhibition Room, Seminar Room), Coffee break (-1 level)
17:00 – 18:00	ISFG General Session Talks (Large Hall)
18:00 – 19:00	ISFG General Assembly (Large Hall)

Friday, September 4, 2015

09:00 – 09:45	lenary lecture: Tomasz Grzybowski (Large Hall)
09:45 – 10:30	ISFG General Session Talks (Large Hall)
10:30 – 11:30	Poster viewing (Exhibition Room, Seminar Room), Coffee break (-1 level)
11:30 – 13:00	ISFG General Session Talks (Large Hall)
13:00 – 14:30	Lunch Break (-1 level), Qiagen Symposium (Medium Hall A&B)
14:30 – 16:00	ISFG General Session Talks (Large Hall)
16:00 – 16:30	Coffee break (-1 level)
16:30 – 17:30	ISFG General Session Talks (Large Hall)
19:00 – 00:00	Congress Dinner (Zalesie Manor Complex)

Saturday, September 5, 2015

09:00 – 10:30	ISFG General Session Talks (Large Hall)
10:30 – 10:45	Coffee break (-1 level)
10:45 – 11:30	ISFG General Session Talks (Large Hall)
11:30 – 12:00	Closing Ceremony (Large Hall)

www.isfg2015.org