www.isfg2017.org

SEOUL

27th Congress of the International Society for Forensic Genetics

August 28 - September 2, 2017 | COEX

ISFG 2017

27th Congress of the International Society for Forensic Genetics

August 28 - September 2, 2017
Seoul, Republic of Korea

Hosted by

검찰
PROSECUTION SERVICE

Supreme Prosecutors' Office, Republic of Korea

ISFG

International Society for Forensic Genetics

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ISFG 2017 SEOUL

Welcome Message

GREETINGS FROM THE CONGRESS PRESIDENT

A Leaping-Forward Step in Forensic Genetics

It is a great pleasure for us to invite leading forensic genetic scientists from all over the world for the “27th International Congress of International Society for Forensic Genetics (ISFG)," the Society that has the longest history and the greatest prestige in the field of forensic genetics.

It is quite well known that investigation technologies using forensic genetics including offender DNA database play a huge role in the criminal justice system, not only in the Republic of Korea, but around the world. Now that forensic genetics is moving into the next phase driven by the rapid development of life science, this Congress will be a venue to provide the participants with valuable lectures from invited keynote speakers and workshops. Vibrant discussions are expected to be made on various and important topics such as utilization of research results and the consequential ethical conundrums from human genome studies, and the need to establish a developed model for mixed DNA interpretation which poses one of tricky issues in individual identification. In particular, this Congress, which is held in Asia for the first time, will be marked as a meaningful occasion if it becomes a start to vitalize joint research between the East and the West, looking into genetic diversity. I sincerely hope that this week-long Congress helps the participants to solidify mutual bonds and build new partnerships.

Seoul, where this Congress is held, has been the capital of Korea for more than 600 years since the Joseon Dynasty. In addition, the city has been the center of remarkable economic development over the last 50 years. On top of that, Seoul is a global city that blends the culture of yesterday with the trends of today. It is hoped that all of the participants will actively be engaged in presentations and discussions to set milestones in the development of forensic genetics, and experience Korea's history and culture.

The Organizing Committee will spare no efforts in making the event successful and helping your stay more comfortable. Thank you.

Moon Mooil

Moon, Moo-il
Congress President & Prosecutor General of the Republic of Korea

ISFG 2017 SEOUL

About ISFG

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.

LOCAL 2017 CONGRESS ORGANISING COMMITTEE

Congress President
Prosecutor General
Moon, Moo-Il

Congress Spokesperson
Forensic Research Institute
Lee, SeungHwan

Chairman of LOC
Director General of Forensic Science Investigation Dept.
Lee, Jeong-Hoe

Vice-Chairman of LOC
Chief of Forensic Investigation Planning Office
Jeon, Hyung-keun

Head of Task Force Team
Director of Forensic Science Division II
Chung, Jinyong

Secretary of Task Force Team
Senior DNA Analyst of Forensic Science Division II
Oh, HyeHyun

Local Scientific Committee
Chair **Lee, SoongDeok**
Secretary **Lee, HwanYoung**
Chung, Ki Wha
Jung, Kyu Won
Kim, Chang-Bae
Kim, Seon-Young
Lee, JaeWon
Lee, Seung Hwan
Lee, YangHan
Shin, Kyoung-Jin

Government Sponsors
Ministry of Justice
National Police Agency
National Forensic Service
Ministry of National Defense
Criminal Investigation Command
Ministry of Science and ICT
National Research Foundation of Korea
Korea Tourism Organization
Seoul Metropolitan City

ISFG BOARD AND SCIENTIFIC COMMITTEE

Walther Parson, Innsbruck
Peter M. Schneider, Cologne
John Butler, Gaithersburg
Mechthild Prinz, New York
Leonor Gusmão, Rio de Janeiro

CONGRESS VENUE

Coex, 159 Samseong dong, Gangnam-gu, Seoul, Republic of Korea

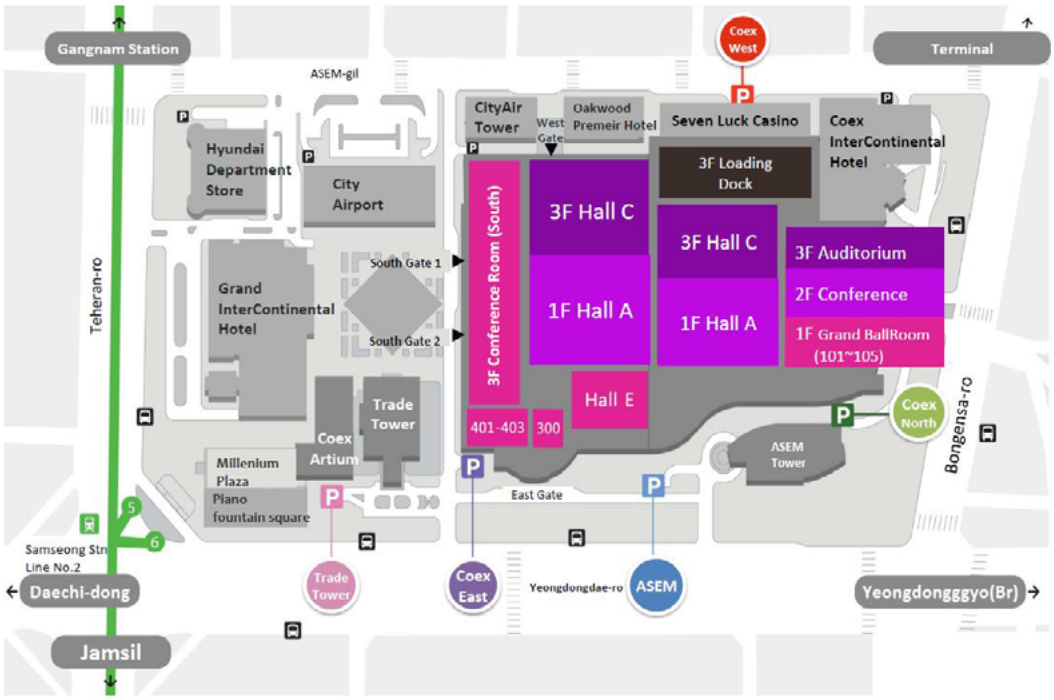
ISFG 2017 SEOUL

Congress Information

CONGRESS VENUE

The Coex Convention & Exhibition Center in Seoul is Korea's premier events venue, providing more than 20 years of experience and know-how in the domestic and international MICE industry. Coex is conveniently located in the heart of Seoul's business district with major 5-star hotels, a huge shopping mall, convenient transportation linkage, and an advanced IT infrastructure to ensure that all you need your needs are met right here under one roof.

The Coex Convention & Exhibition Center is comprised of four stories above ground with a total of 36,007m^2 of exhibition space and a floor area of 460,000m^2. Four specialized exhibition halls can be partitioned into a total of 12 separate rooms, and include a convention hall with space for up to 7,000 people. The Coex Center also boasts 54 meeting rooms and office space equipped with state-of the-art facilities and a cutting edge building management system.



Smoking policy

The entire congress venue is a non-smoking area. The smoking areas are located outside the building.

How to get to the venue

Incheon International Airport

Limousine(Standard) (#6006)	Limousine (Deluxe) (#6103)	Limousine(Deluxe) (#6704)
Approximately 60 min KRW10,000(Adult, one-way) Samseong Station exit 7 Bus Stop	Approximately 65 min KRW16,000(Adult, one-way) City Airport Bus Stop	Approximately 80 min KRW16,000(Adult, one-way) Coex Intercontinental Hotel Bus Stop
Taxi	**Subway**	
Approximately 60 min KRW50,000(standard) KRW90,000(deluxe)	Incheon International Airport Station(Incheon Airport Railroad) > Hongik Univ. Station, transfer to subway line 2 > Samseong Station(Duration – Approx. 101 min) Incheon International Airport Station(Incheon Airport Railroad) > Gimpo Airport Station, transfer to subway line 9 > Bongeunsa Station(Duration – Approx. 111 min)	

Gimpo International Airport

Limousine(Standard) (#6104)	Taxi	Subway
Approximately 45 min KRW7,500(Adult, one-way) City Airport Bus Stop	Approximately 60 min KRW50,000(standard) KRW90,000(deluxe)	Gimpo International Airport Station(line 5) > Yeongdeungpo-gu Office Station, transfer to line 2 > Samseong Station

REGISTRATION

The registration desk is divided into two places depending on the program. The name badge for workshop can be picked up at the registration desk located in front of room 300, Conference Room South (3F). The name badge for ISFG 2017 can be picked up at the registration desk located in the lobby at the Auditorium (3F).

Date	Operation Hours	Location
August 28 (Mon)	08:00 - 18:00	Room 300 Conference Room South 3F (South Wing)
August 29 (Tue)	08:00 - 16:00	
	14:00 - 19:00	Auditorium Lobby 3F (North Wing)
August 30 (Wed)	08:00 - 18:30	
August 31 (Thu)	08:00 - 18:30	
September 1 (Fri)	08:00 - 17:00	
September 2 (Sat)	08:00 - 12:00	

Tourist Information desk will be located beside the registration desk where you can receive information of tourist attractions and join for tour program in a special rate.

※ For identification and security reasons, name badge should be worn and be visible at all time during the congress and at the social events.
※ If you have lost your name badge please visit the registration desk.

Name Badges

The registration badge is an individual official pass for the entry to sessions, lunches, exhibition areas and social events, except gala dinner.

VIP / CONGRESS PRESIDENT / LOC CHAIR / ISFG BOARD	
LOCAL ORGANIZING COMMITTEE	
DELEGATE	
ONE DAY DELEGATE	
ACCOMPANYING PERSON	
EXHIBITOR	
PRESS / STAFF	

Entitlement

Congress registration fee includes: Admission to all congress sessions (August 30 - September 2), opening ceremony, scientific prize lecture, welcome reception, exhibition areas, coffee breaks, closing ceremony, lunches (August 30 - September 1) and congress material. The workshops (August 28 - August 29) and gala dinner are not included in the registration fee.

Accompanying registration fee includes: Admission to opening ceremony, scientific prize lecture, welcome reception, exhibition areas, coffee breaks, closing ceremony and lunches (August 30 - September 1). The workshops/sessions and gala dinner are not included in the registration fee of accompanying person.

Internet Lounge & Free WIFI

Laptops will be located in the lobby at the Auditorium for Delegate's convenience. Also Coex Free Wi-Fi is available in the congress rooms during the congress

ISFG Working Group Meeting

Date	Meeting	Time	Location
August 30 (Wed)	Asian DNA Group	18:30 - 21:30	Auditorium, 3F
	Chinese Group	18:30 - 19:30	Room 2BC, 3F
	English Group		Room 1BC, 3F
	French Group		Room 1A, 3F
	Italian Group		Room 210, 2F
	DNA Commission		Room 2A, 3F

General Assembly of the ISFG

Date	Meeting	Time	Location
August 31 (Thu)	For ISFG Members only	18:30 - 19:30	Auditorium, 3F

Coffee Break

Coffee is served in Auditorium lobby and exhibition areas temporarily and in the poster sessions at all times.

Date/Time	August 28 (Mon)	August 29 (Tue)	August 30 (Wed)	August 31 (Thu)	September 1 (Fri)	September 2 (Sat)
Morning	11:00 - 11:30	11:00 - 11:30	10:30 - 11:30	10:30 - 11:30	10:30 - 11:30	10:00 - 10:15
Afternoon	16:00 - 16:30	16:00 - 16:30	16:00 - 17:00	16:00 - 17:00	16:00 - 16:15	-

Social Program (Included)

All delegates and accompanying persons are invited to attend the welcome reception after opening ceremony at Grand Ballroom 103+104, 1F (North Wing), Coex. You will have the opportunity to enjoy an amazing entertainment and a typically Korean buffet during the reception. Please don't miss out the chance to join the wonderful events and build social networks.

Program	Date	Time	Location
Welcome Reception (Get Together Party)	August 29 (Tue)	20:00 - 21:30	Grand Ballroom 103+104 1F (North Wing)

Social Program (Optional)

Only the participant who purchased the gala dinner ticket can attend the gala dinner and after-party. The ticket price includes one-free drink at the reception desk at the Theater lobby, Grand Walkerhill Seoul.

Ground transportation from Coex to the Theater will be provided from 18:00 in due to order and return buses will be provided as well from 22:00. When you get on the bus to the gala dinner please show your ticket to the staff.

Program	Date	Time	Location
Gala Dinner	September 1 (Fri)	18:30 - 20:30	Theater, Grand Walkerhill Seoul
After-Party		20:30 - 22:00	Aston House, Grand Walkerhill Seoul

※ Dress Code: Semi-Formal
※ Additional bus will be arranged to Aston House after the Gala dinner at Theater, Grand Walkerhill Seoul for the after-party.

Preview Room for Presenter

The preview room is located in room 1BC (next to the auditorium) for delegates to preview their presentation materials.

Photography

Professional photographers are hired for the congress. The photos may be used for reports, marketing and supplied to the media if requested. These photos will be also uploaded in ISFG 2017 website after the congress. If you don’t agree to be taken in the picture please notify the registration desk.

Mobile Phones

Mobile phones needs to be switched vibrate mode during all the session.

Message

Important notification will be available to check at the congress mobile application. Please refer to the mobile application page for the instruction.

My Coex

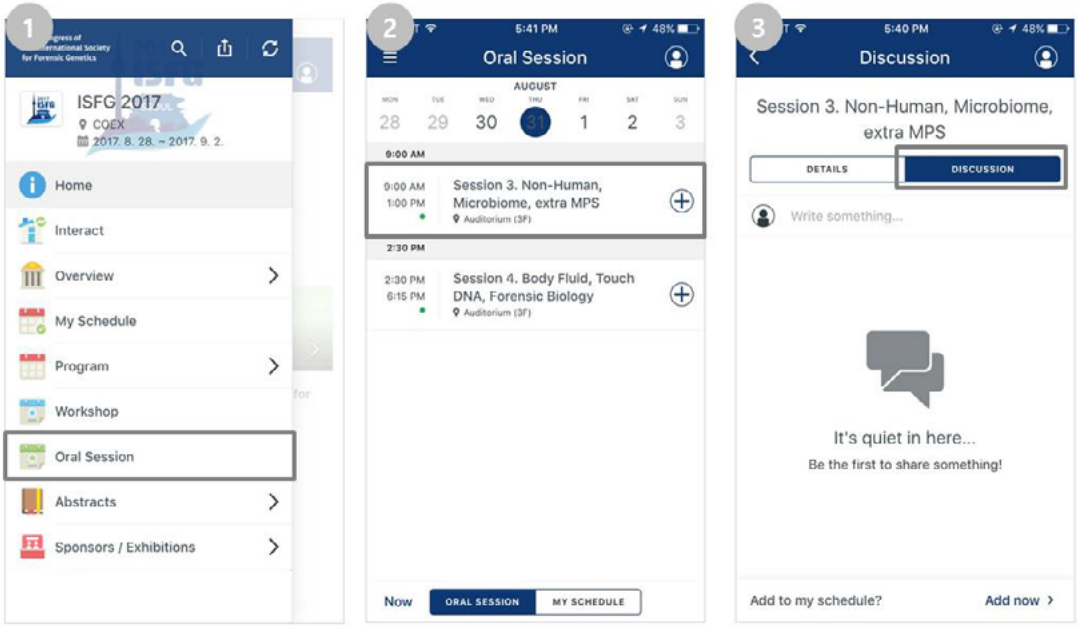
This is a useful app for first-visitor which provides information about restaurants, café, shopping area and city airport terminal in the Coex (B1).

MOBILE APPLICATION

The ISFG 2017 has a mobile application! We strongly encourage you to download our mobile application to enhance your experience at ISFG 2017. You'll be able to plan your day with schedule and browse up-to-date program, maps and pop-up messages. Especially you can ask to the speaker your questions in real time. The session chairs and speaker will select the uploaded questions and answer it. For more information please refer to below instruction.

How to ask questions with the app in Q&A sessions

1) Tap the menu button in the upper left corner of the app
2) Go to the 'Oral Session' menu and click the session which you want to upload questions
3) Click the 'Discussion' menu and post your question with the presenter name

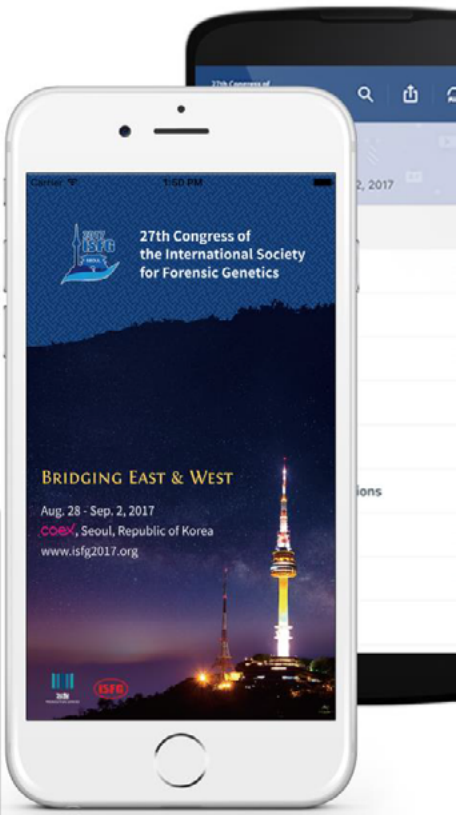


ISFG 2017
(27th Congress of the International Society for Forensic Genetics)
has gone mobile!

Get the app on your mobile device now, for free.

http://guidebook.com/app/ISFG2017/

1. Visit the above URL on your device or Scan QR code
2. Tap the "download" button to get the free app
3. Open the app and download the "ISFG 2017"



Available on

LUNCH

Workshop (Lunch)

For your convenience, priority arrangements have been made with 3 restaurants including restaurants for delegates who have special dietary requirements. The restaurants are located in Coex malls (B1), which are a landmark in Seoul and the largest underground shopping mall in Asia. You can enjoy and experience a variety of options for your lunch during the congress.

Lunch coupons will be included in the name badge. Please have your coupons and hand in to the counter at the restaurant. Lunch coupon will cover up to KRW13,000. Additional cost needs to be paid by itself. Please note these lunch coupons cannot be reissued.

Date	Time	Location
August 28 (Mon)	13:00 - 14:00	CJ Food World, Agra, Kervan Coex Mall (B1), Coex
August 29 (Tue)		

※ This lunch coupons can be used only in 3 designated restaurants.

Lunch Seminar (Lunch)

For the pre-registrant: The lunch will be held in Grand Ballroom 103+104 (1F) next to the poster session room after lunch seminar (13:00 - 13:40).

Date	Time	Location
August 30 (Wed)	13:40 - 14:30	Grand Ballroom 103+104 1F (North Wing)
August 31 (Thu)		
September 1 (Fri)		

※ This lunch seminar coupon is not available at restaurants located in Coex Mall (B1), Coex.

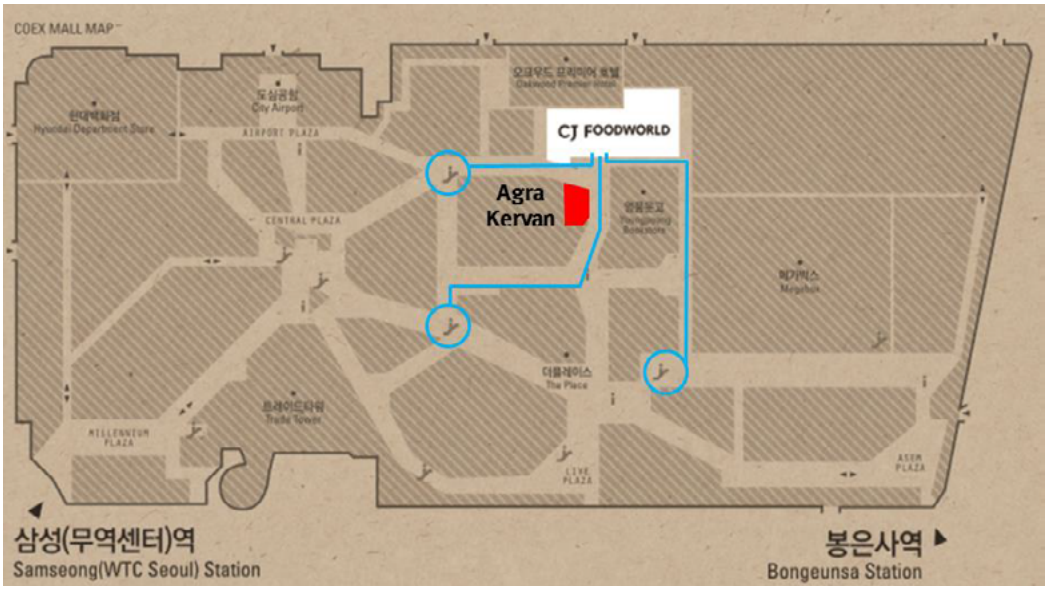
For the non-registrant: Lunch coupon will cover up to KRW13,000. Additional cost needs to be paid by itself. The Lunch coupon can't be overlapped with the lunch coupon of lunch seminar. Please note these lunch coupons cannot be reissued and refer to the information of workshop (Lunch) above.

Date	Time	Location
August 30 (Wed)	13:00 - 14:30	CJ Food World, Agra, KERVAN Coex Mall (B1), Coex
August 31 (Thu)		
September 1 (Fri)		

※ This lunch coupons can be used only in 3 designated restaurants.

[Restaurant Information]

- CJ Food World (Asian food)
- Agra (Indian food) & Kervan (Turkish food)



GUIDELINE FOR ORAL AND POSTER PRESENTER

The ISFG board and the local organizing committee have carefully reviewed a lot of high quality abstracts. All authors of oral and poster presenter are kindly required to follow the instruction as below.

Oral Presentation: All presenters should prepare the presentation file in Microsoft PowerPoint 2013 or Adobe-PDF format in English. All speakers are kindly requested to provide their presentation materials such as font files, original movie files in WMV (Window Media Video). Presenters are also asked to arrive at the Auditorium at least 10 minutes prior to the session begins. Presenters are strongly recommended to use venue facilities. However if you want to use your own devices, please bring all the necessary adaptors, which are compatible with our beam projector (RGB Port Only). It is highly recommended that presenters should hand in their presentation file to the technician who is in the technical room behind the conference room.

[Presentation Time]

- Keynote presentation: 45 minutes (including Q&A time)
- General presentation: 15 minutes (including Q&A time)

Poster Presentation: All posters will be presented in Grand Ballroom 101+102 located on the first floor (North Wing) from August 30 (Wed) to September 1 (Fri). We encourage you to set up your poster as early as possible before August 30 (Wed) 10:00 and to remove your poster after September 1(Fri) 13:00. Poster will be attached at the right position on the designated poster board and the tapes will be provided in the poster session room.

[Poster Presentation Schedule]

- Date : August 30 (Wed) - September 1 (Fri), 2017
- Preparation : August 30 (Wed), 08:00 - 10:30
- Removal : September 1 (Fri), 13:00 - 17:00
- Location : Grand Ballroom 101+102 located on the first floor (North Wing)
- Poster Presentation Schedule

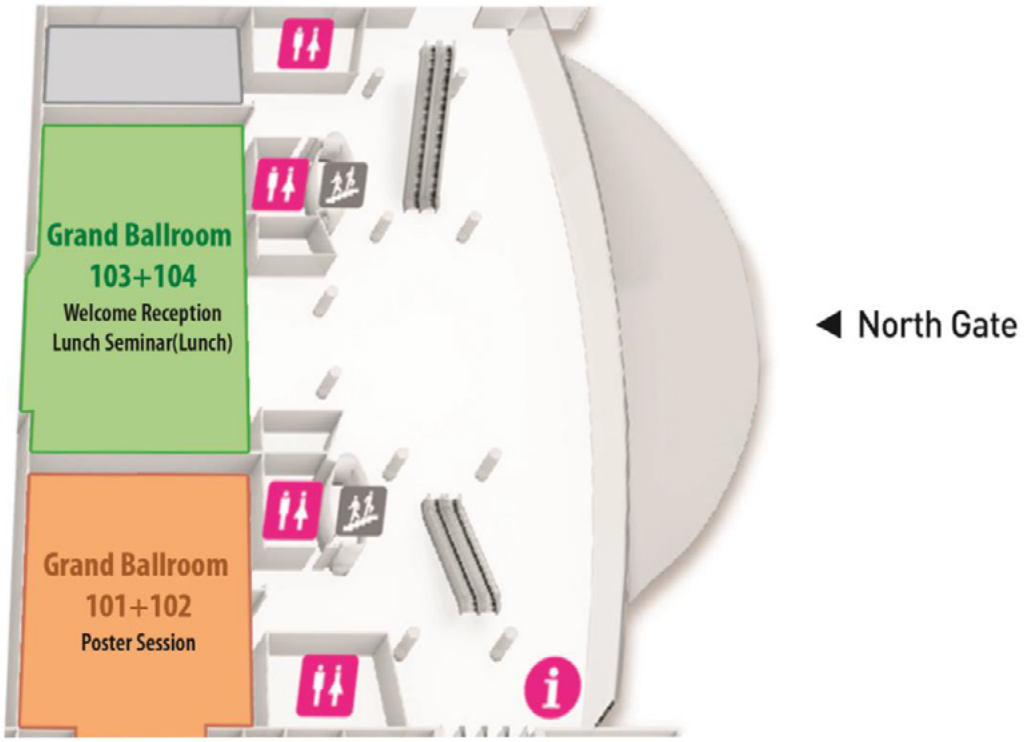
Date/Time	Morning (10:30 - 11:30)	Afternoon (16:00 - 17:00)
August 30 (Wed)	[Session 1] P01 Massively Parallel Sequencing P02 Non-Human, Microbiome	[Session 2] P03 Population Genetics, Lineage Markers, Ancestry, X-Chromosome Variation, Paternity
August 31 (Thu)	[Session 3] P04 New Technologies, DNA Typing Methods, Quality Control	[Session 4] P05 Body Fluids Identification, Touch DNA, Forensic Biology, Legal Medicine
September 1 (Fri)	[Session 5] P06 Ethics & Legal P07 Predictive Makers	-

※ Posters left behind after 17:00, September 1 (Fri) will be discarded without notice.

※ Organizing committee is not responsible for any loss or damage to your poster if it is not removed by the notified time.

ROOM ALLOCATION

1st Floor (North Wing)



Grand Ballroom 101+102
- Poster Session

Grand Ballroom 103+104
- Welcome Reception
- Lunch Seminar (Lunch)

North Gate
- Bus Station (Gala Dinner)

ROOM ALLOCATION

3rd Floor (South Wing & North Wing)

Conference Room South 300, 301, 307
- Workshop (1, 2, 3, 4, 5, 6, 8, 9, 10, 11)

Conference Room South 300
- Registration Desk (Workshop)

Conference Room South 304
- KIT Desk

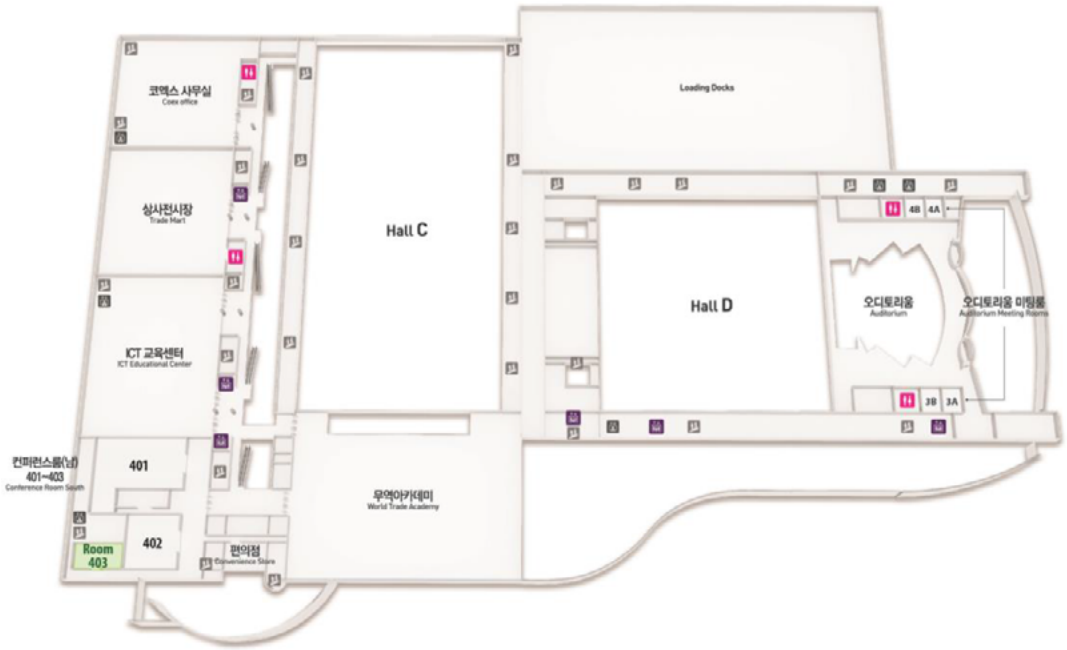
Conference Room South 305
- ISFG Board Meeting

Prayer Room (Nearby Hall E)

Auditorium
- Registration Desk (ISFG 2017)
- KIT Desk
- Opening Ceremony
- Scientific Prize Lecture
- Session
- Lunch Seminar
- General Assembly of the ISFG
- Closing Ceremony
- Exhibition

ROOM ALLOCATION

4rd Floor (South Wing)



Conference Room South 403
- Workshop (7)

ISFG 2017 SEOUL

ISFG Scientific Prize Lecture & Keynote Speakers

ISFG SCIENTIFIC PRIZE LECTURE

Thomas Parsons / USA

International Commission on Missing Persons(ICMP)

Thomas Parsons is the Director of Science and Technology at the International Commission on Missing Persons (ICMP). He supervises a large technical staff in a multidisciplinary approach to location and identification of the missing through imagery, forensic archaeology, anthropology, pathology, bioinformatics and high throughput DNA analysis. The ICMP has assisted with the DNA-based identification of nearly 20,000 persons, and works on missing persons cases globally. Dr. Parsons has coordinated provision of extensive DNA and other forensic science evidence to the International Criminal Tribunal for Yugoslavia (ICTY) and other courts, and testified on multiple occasions for the ICTY in The Hague. Prior to joining the ICMP, Dr. Parsons worked at the US Armed Forces DNA Identification Laboratory (AFDIL) since August of 1994, and held the position of AFDIL Chief Scientist since 2000. For two years after the 9/11 attacks he served on a seminal National Institute of Justice advisory panel for the World Trade Center DNA identification efforts. His undergraduate degree was in Physics from the University of Chicago, and he received a Ph.D. in Biochemistry from the University of Washington in 1989. As a postdoctoral fellow at the Smithsonian Institution, he focused on ancient DNA, molecular evolution and phylogenetics, as well as mtDNA biogeography and avian speciation.

KEYNOTE SPEAKER

Kyoung-Jin Shin / Republic of Korea

Yonsei University College of Medicine

Kyoung-Jin Shin studied dentistry at Yonsei University, Seoul, Korea. He earned his M.S. in Forensic Odontology at Yonsei University in 1999, and received his Ph.D. in Forensic Genetics at Yonsei University in 2002. He is currently professor and Chair of the Department of Forensic Medicine, Yonsei University College of Medicine.

His early work in forensic genetics was focused on DNA typing of old skeletal remains. He has participated in the national projects for a long time such as tracing casualties from Korean War through DNA typing and genetic analysis of mummies from several medieval tombs. His intensive efforts in the analysis of sequence variations and phylogeny of mtDNA have led to the development of a web-based forensic bioinformatics resource, mtDNAmanager (http://mtmanager.yonsei.ac.kr).

Other topics of interest include the population genetics of Y-chromosomal STR/SNP in Koreans and its application to forensic genetic investigations. He designed also prototypes of Koreanized commercial STR multiplex PCR systems, Kplex Kit series. More recently, he has developed NGS panels for forensic analysis of mitochondrial DNA, autosomal/Y-chromosomal STR and microhaplotype markers.

Mark Jobling / UK

University of Leicester

Mark Jobling studied Biochemistry at the University of Oxford and undertook a PhD there, before moving to the Department of Genetics of the University of Leicester. He is an evolutionary geneticist interested in the pattern and process of human genome variation. He has specialized in Y-chromosome diversity, exploiting this male-specific marker in studies of population history, sex-biased admixture, mutation processes, forensic analysis and genetic genealogy. Mark's current research projects focus on the use of next-generation sequencing in population genetics and forensics, and include work in non-human species such as great apes.

Mehrdad Hajibabaei / Canada

University of Guelph

Dr. Mehrdad Hajibabaei is an expert in evolutionary/environmental genomics and bioinformatics. His research has focused on the use of genomics information in biodiversity analysis, ranging from the elucidation of deep branches of the tree of life to the establishment and application of marker genes (DNA barcodes) for species identification. Hajibabaei has been one of the pioneers in the use of high-throughput genomics technologies, such as Next-Generation Sequencing (NGS) for the assessment of biodiversity in samples as varied as bulk environmental water, soil and sediments to food and natural health products. Hajibabaei has played a leadership role in establishing and managing large-scale research projects and collaborative networks such as the Canadian Barcode of Life Network and the International Barcode of Life (iBOL). Since 2011, Hajibabaei has been leading Biomonitoring 2.0 (www.biomonitoring2.org), a large-scale applied genomics project involving seven research groups. This project uses NGS technologies for comprehensive assessment of biodiversity in Canada's priority ecosystems including regions exploited for Oil Sands operations. Hajibabaei has served on advisory and review panels for major initiatives (iBOL, NEON), international organizations (e.g. IUCN) and funding agencies, and has collaborated with regulatory agencies (e.g. US EPA, US FDA, Environment Canada, Natural Resources Canada, Parks Canada) and various industries. Hajibabaei is currently the Associate Editor of four scientific journals. He has trained 9 Postdocs and 11 graduate students and his research publications have generated 7828 citations with an h-index of 34.

Titia Sijen / Netherlands

Netherlands Forensic Institute

Titia Sijen, PhD (female, 1967, H-index=28, i10-index=47) leads the Biological Traces research team of the NFI since 2007. Before, Titia studied quite successfully RNA silencing processes in both plants and nematodes and her experience with RNA molecules may have fed Titia's interest in forensic RNA analysis. In addition, her team studies the analysis and (probabilistic) interpretation of complex DNA profiles, develops tools for bloodstain pattern analysis, optimizes mitochondrial DNA typing and explores the application of massively parallel sequencing for forensic purposes. Besides technological improvements, much effort is spent on developing interpretation strategies and ensuring full validation and implementation for forensic casework so that methodologies are correctly and effectively used by forensic practitioners.

Manfred Kayser / Netherlands

Erasmus University Medical Center Rotterdam

Manfred Kayser currently is Professor of Forensic Molecular Biology and Head of the Department of Genetic Identification (formerly known as Dept. of Forensic Molecular Biology) at Erasmus University Medical Center Rotterdam. He received his diploma in biology from University of Leipzig in 1994, his Ph.D. in biology/genetics with summa cum laude from Humboldt University Berlin in 1998, and his habilitation in genetics from University of Leipzig in 2004. After postdoctoral research at the Department of Anthropology, Pennsylvania State University, he was staff scientist, and later Heisenberg Fellow of the German Research Council, at the Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology Leipzig, before his full professorship appointment at Erasmus University in 2004. His research interest is in various aspects of forensic genetics and anthropological genetics. In the forensic world, he is well known for the introduction and further development of forensic Y-chromosome analysis, and his pioneering work on establishing Forensic DNA Phenotyping as new subfield. He (co)authored >200 articles in peer-reviewed scientific journals, books, and encyclopedias (H factor >50), serves as academic editor, editorial board member and ad hoc reviewer for several journal, and regularly accepts invitations to present at international conferences and institutes worldwide.

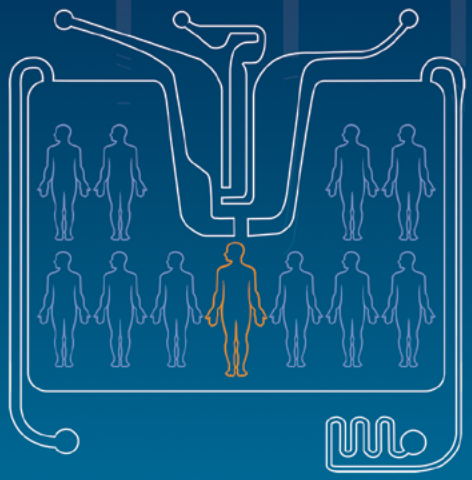
Susan Friedman / USA

The Innocence Project

Susan Friedman is a staff attorney at the Innocence Project and litigates post-conviction DNA cases throughout the country. She also supervises students through the Innocence Project clinic at Cardozo Law School. Ms. Friedman joined the Innocence Project as a staff attorney in December 2015. Before that, Ms. Friedman was a staff attorney with the DNA Unit at the Legal Aid Society in New York where her practice focused on emerging DNA technology. From 2011-2013, Ms. Friedman was a staff attorney and Equal Justice Works Fellow with the Mid-Atlantic Innocence Project. Ms. Friedman graduated from Mount Holyoke College in 2005 and from The George Washington University Law School in 2011. She also holds an M.S. in Biomedical Sciences from Mount Sinai School of Medicine Graduate School of Biology.

PRECISE. FORENSIC. EVIDENCE.

DIGITALLY RESOLVING HETEROGENEITY IN BIOLOGICAL MIXTURES



DEPArray™ NxT

With the ability to analyze even minute mixed biological evidence, the DEPArray NxT system can separate epithelial cells, white blood cells, and sperm cells, and recover 100% pure pools of cells for standard forensic analysis profiles produced from these separated samples. This represents a fundamental step in assigning the precise and distinct genetic fingerprints from each contributor.

DEPArray™ NxT Image Based Cell Sorting with Single-Cell Precision

Visit us at ISFG 2017 | Booth A02

MENARINI silicon biosystems

www.siliconbiosystems.com/forensics

ISFG 2017 SEOUL

Workshops Program

Promega

Discover the CE you've been missing

Spectrum CE SYSTEM

Come on a journey of discovery to see what the Spectrum CE System can do for you.

www.promega.com/CE

WORKSHOPS

Workshop 1

Topic	Probabilistic Genotyping Workshop on The Use of the Open Source Software LRmix Studio and EuroForMix
Organizer(s)	Peter Gill, Corina Benschop, Øyvind Bleka
Presenters	Peter Gill, Corina Benschop, Øyvind Bleka
Type	Hands on (Seating capacity : 50)
Date	August 28 (Mon)
Time	09:00 - 18:00 (Coffee Break : 11:00 - 11:30 / 16:00 - 16:30)
Location	Conference Room South 301A (3F, Coex)
Description	This workshop will introduce the principles behind mixture interpretation using the most up-to-date methods that are currently available. The likelihood ratio method will be described and participants will be shown how to formulate propositions. The principles of mixture interpretation where there is drop-out and drop-in will be described. Then the guidelines of the ISFG DNA commission on the evaluation of mixtures and software validation will be discussed. Two different free open-source software will be demonstrated: LRmix Studio http://lrmixstudio.org/ and EuroForMix http://www.euroformix.com/. The former is a qualitative model, whereas the latter is quantitative, taking account of peak height, stutter and degradation to formulate the likelihood ratio. Both are fully validated according to the ISFG DNA Commission recommendations. The differences between the two software, the expected trends as well as statement writing will be discussed. Finally, there will be practical demonstration of the models. Participants are expected to bring their own laptops and to download the necessary software so that they can run exercises and analyse their own cases. At the end of the workshop participants will be proficient in both software approaches.

Workshop 2

Topic	Beyond DNA Profiling : On Other Forensic Questions than 'Who is it?'
Organizer(s)	Titia Sijen, Roland Van Oorschot, SallyAnn Harbison
Presenters	Roland van Oorschot, SallyAnn Harbison, Titia Sijen, Athina Vidaki, Hwang Young Lee, Bianca Szkuta
Type	Lecture (Seating capacity : 100)
Date	August 28 (Mon)
Time	09:00 - 18:00 (Coffee Break : 11:00 - 11:30 / 16:00 - 16:30)

Location	Conference Room South 307 (3F, Coex)
Description	Although human DNA profiling has the potential to present strong evidence for placing a suspect at a crime scene, forensic questions that go beyond 'Who is it?' are increasingly asked. Information of the type of cell material ('What is it?') and on possible modes of deposition ('How did it get there?') may assist forensic interpretation on the activity level. In the workshop, we will discuss techniques such as RNA profiling and DNA methylation that enable cell type inference. Casework examples will illustrate the possibilities and participants will actively work with exemplar results. Besides, we will elaborate on the prevalence of background cell material and factors that have a role in transfer and persistence. Interactive sessions and exercises will illustrate the possibilities and pitfalls regarding data interpretation that may extend to the activity-level. The workshop will have multiple presenters (besides the workshop organizers, specialists on DNA methylation and activity-level aspects will be included), and a variety of formats to provide hands-on experience with all subjects and interaction between workshop presenters and participants.

Workshop 3

Topic	Making Sense of Ethical & Social Aspects of Forensic Genetics
Organizer	Matthias Wienroth
Presenter	Matthias Wienroth
Type	Lecture (Seating capacity : 50)
Date	August 28 (Mon)
Time	09:00 - 13:00 (Coffee Break : 11:00 - 11:30)
Location	Conference Room South 301B (3F, Coex)
Description	The aim of the workshop is to make accessible to forensic stakeholders the concept of 'ethical and social aspects' of technology by exploring a case study in the forensic genetics field. We will not discuss ethics approval procedures or health & safety concerns, but instead look at the type of knowledge that ethical and social aspects of science represent. By introducing the notion of 'anticipatory governance,' workshop participants will be equipped to explore how such understanding can be applied to enhance forensic genetics research and technology uses. Objective 1: to enable participants to commence acquiring some of the tools necessary for assessing ethical and social aspects of the use of forensic genetics technologies, especially for those that may not yet be regulated (e.g. Forensic DNA Phenotyping; Familial Searching; Massively Parallel Sequencing; inference of clinically relevant information). Objective 2: to encourage participants to locate themselves as researchers, and their capacity to act, within the complex system of technology governance, in order to support the management of dilemmas in researching and applying new/emerging technologies.

Workshop 4

Topic	Y Chromosome: Sequence variation, Population database, Frequency estimation, Mixture interpretation, Kinship analysis, Ancestry inference
Organizer	Lutz Roewer
Presenters	Lutz Roewer, Sascha Willuweit
Type	Hands on (Seating capacity : 100)
Date	August 28 (Mon)
Time	09:00 - 13:00 (Coffee Break : 11:00 - 11:30)
Location	Conference Room South 300 (3F, Coex)
Description	The Y chromosome is a multiple-purpose marker with ever increasing importance in forensic casework provided that samples are properly analyzed and results are correctly interpreted and reported. The main applications comprise (1) the analysis of crime scene evidence especially mixed female-male traces, (2) the analysis of paternal genealogies and (3) the inference of biogeographical ancestry in cases of missing persons or unknown suspects. All applications benefit from the availability of high-resolution Y-STR kits and efficient protocols to analyze Y-SNPs. The interpretation and decision-making in all three fields is supported by the YHRD (https://yhrd.org), an online facility which provides massive amounts of reference data and state-of-the-art analysis tools. The workshop participants will learn how frequencies for Y profiles will be calculated using constant and variable estimators. The influence of population substructure on the estimates will be demonstrated. Likelihood-based approaches to analyze mixtures and solve kinship cases are another important topics of the workshop program.

Workshop 5

Topic	Scientific Publications : Reading, Writing, and Reviewing
Organizer	John Butler
Presenter	John Butler
Type	Lecture (Seating capacity : 100)
Date	August 28 (Mon)
Time	14:00 - 18:00 (Coffee Break : 16:00 - 16:30)
Location	Conference Room South 300 (3F, Coex)
Description	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. 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 workshop 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 and examined.

Workshop 6

Topic	Mitochondrial DNA : EMPOP, MPS, Degraded DNA
Organizer(s)	Walther Parson, Catarina Xavier
Presenters	Walther Parson, Catarina Xavier
Type	Hands on (Seating capacity : 50)
Date	August 28 (Mon)
Time	14:00 - 18:00 (Coffee Break : 16:00 - 16:30)
Location	Conference Room South 301B (3F, Coex)
Description	This workshop provides a brief overview of mitochondrial DNA analysis in forensic genetics using practical casework examples. This year, we focus on the analysis of degraded DNA using Massively Parallel Sequencing (MPS), its link to EMPOP and the new EMPOP search and alignment tool SAM. Participants are made familiar with the new functionalities 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 (MPS) mtDNA data - searching and interpreting point and length heteroplasmy - estimating the haplogroup status of an mtDNA sequence - interpreting geographic distribution of haplotypes and haplogroups Participants may send questions on databasing/database use/EMPOP beforehand. Please send to walther.parson@i-med.ac.at.

Workshop 7

Topic	The Sequencing of STR and SNP Marker Panels Applied to Forensic Genetics
Organizer(s)	Chris Phillips, Peter Vallone
Presenters	Peter Vallone, Chris Phillips, Katherine Gettings, Runa Daniel, David Ballard, Laurence Devesse, Sarah Riman
Type	Lecture (Seating capacity : 100)
Date	August 29 (Tue)

Time	09:00 - 18:00 (Coffee Break : 11:00 - 11:30 / 16:00 - 16:30)
Location	Conference Room South 403 (4F, Coex)
Description	The availability of forensically-relevant sequencing technology platforms along with focused STR and SNP marker panels is enabling new areas of research and applications for forensic casework. The potential of routine and robust sequencing of hundreds of markers opens up the promise of new information for the forensic practitioner. The workshop will include talks in the areas of: STR sequence data generated from various populations, the potential of microhaplotype marker systems, performance of ancestry prediction markers, updates on STR nomenclature, enhanced resolution of DNA mixtures, and considerations for the validation of sequencing methods. There will a special emphasis on East Asian populations throughout the workshop. Attendees should be generally familiar with the current sequencing platforms and chemistries as these will not be a focus of the workshop.

Workshop 8

Topic	Predictive DNA Analysis in Forensic Genetics
Organizer(s)	Manfred Kayser, Wojciech Branicki
Presenters	Manfred Kayser, Wojciech Branicki, Christopher Phillips, Ewelina Pospiech, Magdalena Kukla-Bartoszek
Type	Lecture (Seating capacity : 100)
Date	August 29 (Tue)
Time	09:00 - 18:00 (Coffee Break : 11:00 - 11:30 / 16:00 - 16:30)
Location	Conference Room South 300 (3F, Coex)
Description	In this one-day workshop, participants will be introduced to the concept, principles, markers, models, and methods of predicting appearance, age, and ancestry (i.e. Forensic DNA Phenotyping) in forensic genetics. Participants will learn about the theoretical basis of predictive DNA analysis, including the identification and selection of DNA markers, prediction modeling and interpretation of prediction outcomes. The workshop will provide an overview of markers, methods and tools currently available for predicting appearance, age, and ancestry from DNA and the practical value of forensic predictive DNA analysis. Distinctive properties of DNA methylation markers and their suitability for predicting age will be demonstrated. Participants will learn about different technologies for genotyping appearance or ancestry predictive SNPs (such as SNaPshot and massively parallel sequencing) as well as about quantitative methods for DNA methylation analysis suitable in forensics. At the end of the workshop, participants will have an overview about current possibilities and future perspectives in the field of Forensic DNA Phenotyping, and will be able to understand strengths and weaknesses of selected currently available predictive DNA systems.

Workshop 9

Topic	Kinship Analysis
Organizer(s)	Thore Egeland, Klaas Slooten
Presenters	Thore Egeland, Klaas Slooten
Type	Hands on (Seating capacity : 50)
Date	August 29 (Tue)
Time	09:00 - 18:00 (Coffee Break : 11:00 - 11:30 / 16:00 - 16:30)
Location	Conference Room South 301A (3F, Coex)
Description	The workshop will focus both on the theory and on the applications of genetic kinship testing using forensic DNA profiles. We will start with the basic principles of inheritance and how likelihoods for pedigrees can be calculated, explaining various algorithms. These likelihoods are needed for likelihood ratio (LR) calculations and we will discuss important properties of likelihood ratio tests in general. This includes a discussion of the distributions of the LR that one can expect, and how to use and interpret these distributions including possible interpretational pitfalls. Applications of kinship analysis such as Disaster Victim Identification and Familial Searching involve very large numbers of comparisons. We will discuss the particularities of these applications, such as the possible search strategies, choice of mutation model, ways to handle partial or mixed profiles affected by allelic dropout, and linkage. These applications are exploiting kinship to solve identification problems. However, relatedness can also play another role: for example, in mixed profiles relatives can be more easily falsely included than random individuals. In this case, relatedness can make identification more difficult. We will therefore discuss the relevance of LR's for mixture calculations involving kinship. The workshop will be a mix of presentations and exercises leaving also ample time for discussion.

Workshop 10

Topic	Autosomal STR Markers and Interpretation
Organizer(s)	John Butler, Lisa Borsuk
Presenters	John Butler, Lisa Borsuk, Katherine Gettings
Type	Lecture (Seating capacity : 50)
Date	August 29 (Tue)
Time	09:00 - 13:00 (Coffee Break : 11:00 - 11:30)
Location	Conference Room South 301B (3F, Coex)

Description	Short tandem repeat (STR) markers have been used by the forensic genetics community since the mid-1990s to produce DNA profiles to answer questions in criminal investigations and relationship testing. Millions of STR profiles have been generated and hundreds of population studies have been published over the past two decades. This workshop will examine the STR markers commonly used by the forensic genetics community along with configurations of commercial STR kits and artifacts produced (e.g., stutter products and non-template addition) that impact interpretation. Sequence variation observed and allele nomenclature approaches will be discussed as will efforts to expand core STR loci used in countries around the world. The content and value of websites, such as STRBase (http://www.cstl.nist.gov/strbase/) and STRidER (http://strider.online/), will also be covered.

Workshop 11

Topic	aDNA : Mass Disaster, Forensic Anthropology (Bone Samples)
Organizer	Thomas Parsons
Presenters	Thomas Parsons, Jodi Irwin, Jodi Ward, Charla Marshall, Turi King, Anne Stone, Yuryang Jang
Type	Lecture (Seating capacity : 50)
Date	August 29 (Tue)
Time	14:00 - 18:00 (Coffee Break : 16:00 - 16:30)
Location	Conference Room South 301B (3F, Coex)
Description	Many applications of forensic genetics deal with degraded DNA in small quantities. This is notably true in the extraction and analysis of DNA from skeletal samples such as bones or teeth. The ability to analyze these challenging samples plays a very large role in human identification, for example from mortal remains recovered from mass graves, disasters, routine missing persons, or historical cases. For bone samples, much of the expertise encompassed in the forensic genetic arena has its origin the field of “ancient DNA.” This is because the preservation capacity of bone samples enables the survival of genetic information in extremely old or extinct specimens; however, the protective bone matrix also poses challenges for recovery of DNA. This workshop will focus on the characteristics of DNA preservation in bone, and specific challenges and solutions to forensic DNA analysis of old, degraded bone samples. This will include newly developing approaches that use massively parallel sequencing, and highlight recent work in ancient DNA that may show the way for improvements in forensic applications.

ISFG 2017 SEOUL

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Lunch Seminar

Lunch Seminar

Lunch Seminar will be held on August 30 - September 1 from 13:00 to 14:30 in Auditorium 3F. In this interactive seminar, participants will have the possibility to discuss highly topical subjects. Only for the pre-registrant may attend the Lunch Seminar.

Thermo Fisher Scientific	
Date	August 30 (Wed)
Time	13:00 ~ 14:30
Location	Seminar: Auditorium, 3F Lunch: Grand Ballroom 103+104
Title	When Worlds Converge™: A New Approach to Analyzing CE and MPS STR Data
Speaker	Sheree Hughes-Stamm (Professor, Sam Houston State University)

Promega Corporation	
Date	August 31 (Thu)
Time	13:00 ~ 14:30
Location	Seminar: Auditorium, 3F Lunch: Grand Ballroom 103+104
Title	Improved Capillary Electrophoresis Systems for Human Identification: The Spectrum CE Systems
Speaker	Lotte Downey (Senior Global Strategic Marketing Manager)

Menarini Silicon Biosystem	
Date	September 1 (Fri)
Time	13:00 ~ 14:30
Location	Seminar: Auditorium, 3F Lunch: Grand Ballroom 103+104
Title	Enabling forensic genetic analysis of biological mixtures through the identification and recovery of pure cells with the DEPArray™ system
Speaker	Jack Ballantyne (PhD , Department of Chemistry) Francesca Fontana(Biology R&D Manager Menarini Silicon Biosystems)

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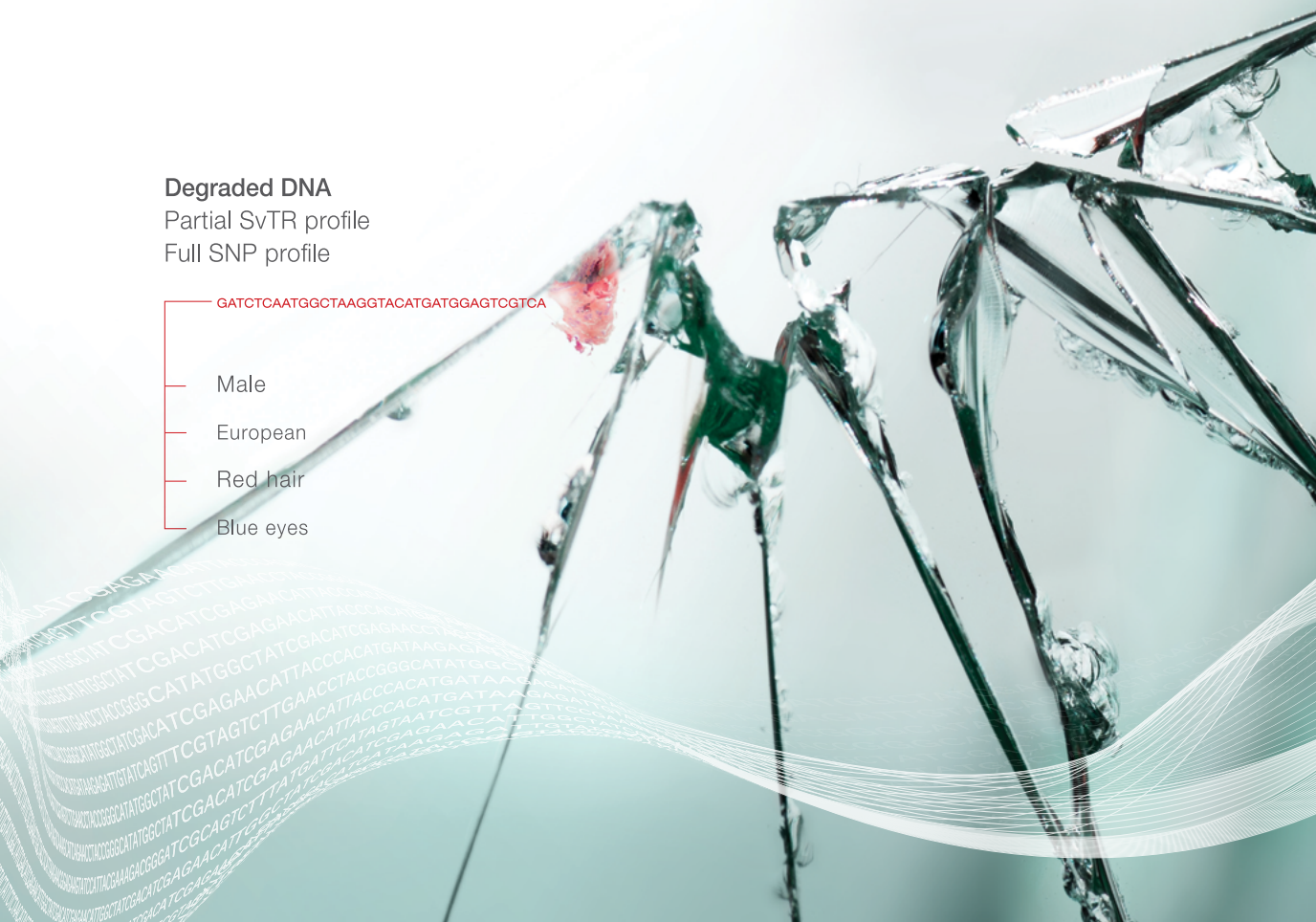
On 30th August at 12.15pm be sure to attend the presentation by Dr Harbison ***"Establishing the limit of detection of massively parralell sequencing using laser micro-dissected cells"*** which will showcase work using the ForenSeq™ DNA Signature Prep Kit and a MiSeq FGx™ Sequencer.

For more information visit the Illumina booth #B-14 & B-15.



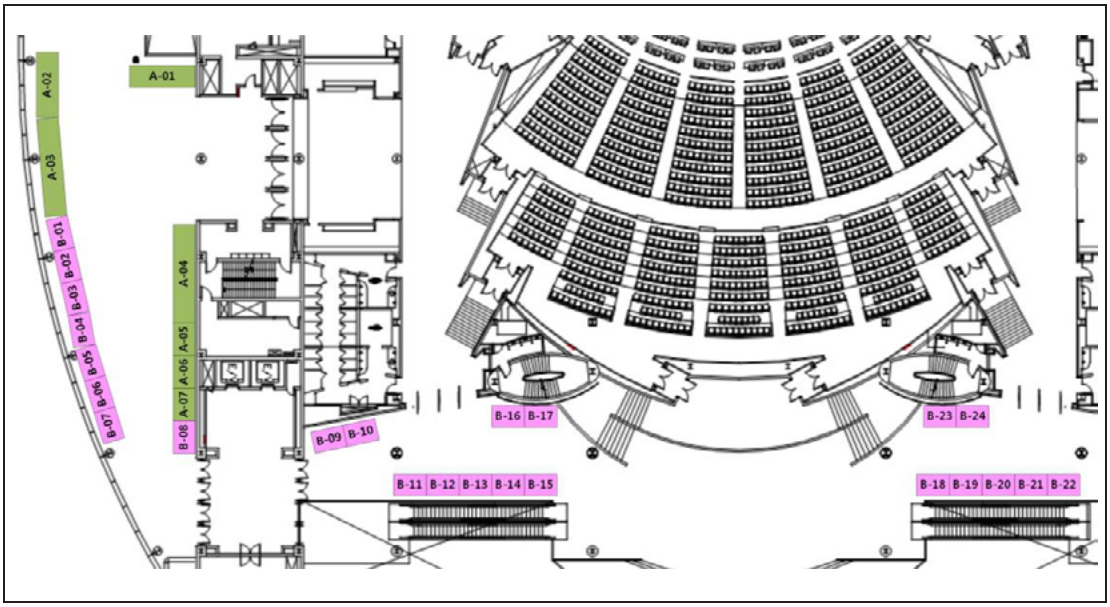
EXHIBITION

The exhibition is located on 3rd Floor of the Auditorium Lobby at Coex. All delegates are invited to visit the exhibition and meet with the company representatives during the Congress.

Exhibition Operating Hours

Date	Time
Wednesday, August 30	08:00 – 17:00
Thursday, August 31	08:00 – 17:00
Friday, September 1	08:00 – 17:00

Booth Allocation



Booth No.	Company Name	Booth No.	Company Name
A-01	QIAGEN	**B-09, B-10**	COPAN
A-02	Menarini Silicon Biosystems	**B-11**	HAMILTON ROBOTICS
A-03	Thermo Fisher Scientific	**B-12**	HEALTH Gene Technologies
A-04	Promega Corporation	**B-13**	KOGENE BIOTECH
A-05	Sercrim Labtech	**B-14, B-15**	ILLUMINA
A-06	DNA Link	**B-16**	ISFG2019 CZECH REPUBLIC
A-07	Eppendorf	**B-17**	Supreme Prosecutors' Office
B-01	GORDIZ	**B-18**	KOMA BIOTECH
B-02	INDEPENDENT FORENSICS	**B-19**	Greenmate Biotech
B-03	AXO SCIENCE - STK	**B-20**	Bio-Medical Science Co., Ltd.
B-04	ADEMTECH	**B-21**	Miltenyi Biotec
B-05	ANDE RAPID DNA	**B-22**	ELSEVIER JOURNALS
B-06	BIONEER Corp.	**B-23**	qualitype GmbH
B-07	LGC-PARADNA	**B-24**	STRmix
B-08	DOUZONE BIZON		

EXHIBITORS INFORMATION

Booth Number: A-01

Company/Institute Name	QIAGEN
Address	5th Fl., Seoul Square 416, Hangang-daero, Jung-Gu, Seoul 100-714, Korea
Website	www.qiagen.com
Tel	080-000-7146

QIAGEN is the leading global provider of Sample to Insight solutions that enable customers to gain valuable molecular insights from samples containing the building blocks of life. Our 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 workflows. QIAGEN provides solutions to more than 500,000 customers around the

world in Molecular Diagnostics (human healthcare), Applied Testing (forensics, veterinary testing and food safety), Pharma (pharma and biotech companies) and Academia (life sciences research).

Booth Number: A-02

Company/Institute Name	Menarini Silicon Biosystems
Address	Via Giuseppe di Vittorio 21b/3 Castelmaggiore 40013 Bologna Italy
Website	www.siliconbiosystems.com
Tel	+39 051 9944100

Menarini Silicon Biosystems is a biotech company, which developed the DEPArray™system, a breaktrough technology that can select, sort and collect both individual and groups of cells from a variety of samples. DEPArray™ technology resolves heterogeneity in biological mixtures of forensic cases and biological evidences. By utilizing a proprietary electronic chip-based microfluidic cartridge and microscope image analysis, the DEPArray™ system works with low input and separates forensic samples composed of epithelial cells, white blood cells, and sperm cells into 100% pure cell type specific preparations, enabling clear-cut genetic profiles.

Booth Number: A-03

Company/Institute Name	Thermo Fisher Scientific
Address	12FL, Suseo Office Building 281, Gwangpyeong-ro, Gangnam-gu, Seoul, Korea
Website	www.thermofisher.com
Tel	+82-2-2023-0600

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Applied Biosystems brand, we help customers accelerate innovation and enhance productivity. As the leading provider in forensic science, we deliver some of the most comprehensively validated products, expertise, and application support available to the human identification community. With 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. thermofisher.com/hid

Booth Number: A-04

Company/Institute Name	Promega Corporation
Address	2800 Woods Hollow Road, Madison, Wisconsin USA
Website	www.promega.com
Tel	608.274.4330

Promega Corporation has provided products for DNA-based human identification for more than 25 years. Please visit our booth to learn what's new from Promega and to see the Spectrum CE Systems in action.

Booth Number: A-05
Company/Institute Name Sercrim Labtech co., ltd.
Address 795-6, Bugaksan-ro, Seongbuk-gu, Seoul, Republic of Korea
Website www.sercrim.com
Tel +82-2-911-4114

Sercrim Labtech co., ltd. which has been supplying the most trustworthy products, is the leading company in Korean lab, scientific & safety solutions market.

Our competitiveness comes from the professionalism built over the last quarter of a century, our close relationsships with global top companies, and the complete trust of our customers. Standing upon those foundations, our business mission is to spur on research and development to be the most reliable solutions partner for customer.

Booth Number: A-06
Company/Institute Name DNA Link, Inc.
Address 2F, Cluster Center, 150, Bugahyeon-ro, Seodaemun-gu, Seoul, Korea
Website www.dnalink.com
Tel 82-2-3153-1500

DNA Link is a company specialized in genomics research, offering accurate experiment analysis and efficient statistical analysis for more than 15 years. We provide customers with top-to-bottom service of highest quality, from appropriate platform and application selection to data analysis.
AccuID® is a whole new SNP based human identification microarray system to genotype hundreds of SNP markers in a single experiment. AccuID® ver. 1.0 consists of markers for SNPs that are ideal for human identification as well as sex determination, specifically, personal identification, maternity/paternity test, and relationship test at 3rd degree relationships even if extremely small amount and degraded DNA.

Booth Number: A-07
Company/Institute Name Eppendorf Korea Ltd.
Address Gala Tower 10F, 46, Nonhyeon-ro 85-gil Gangnam-gu Seoul 06235 Korea
Website www.eppendorf.kr
Tel +82-1577-4395

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide.
Products : pipettes,Liquid Handling system, centrifuges, mixers, spectrometers, PCR cycler, ULT freezers, fermentors, bioreactors, CO2 incubators, shakers, cell manipulation systems.

Booth Number: B-01
Company/Institute Name GORDIZ
Address 143026, Moscow,Russia Skolkovo Innovation Centre, Lugovaya str. 4

building 4
Website www.gordiz.ru
Tel +7 (499) 670-40-41

GORDIZ – innovate biotechnology company. We develop and implements new high-tech solutions for forensic DNA identification in close cooperation with forensic laboratories and scientific research centers. Our highly professional team provides strong technical and methodological support for all customers. Today we can offer our customers a unique range of solutions for all stages of genetic analysis - from extraction and quantification of DNA to DNA fragment and sequence analysis. Our CORDIS line of products for forensic DNA investigation is in use in more than 50 forensic laboratories around the world.

Independent Forensics
DNA Testing & Technologies

Company/Institute Name Independent Forensics
Address 500 Waters Edge, Suite 210, Lombard, IL 60148, USA
Website www.IFI-TEST.com
Tel 1-708-234-1200, skype: dina.mattes1

Solutions for common problems in DNA labs - better body fluid tests for identifying SALIVA, SEMEN, BLOOD, URINE and FECES – faster detection/isolation of sperm from sexual assault evidence using new stain technology and customized microscopes – better collection/recovery of low template DNA samples from Touch DNA, Fingerprints, Adhesives, and Isolated Sperm Cells – a simple to use Post PCR cleanup kit that allows you to use all your data for analysis – a less expensive Multiplex STR kit for use on single source samples. Independent Forensics is a 17025 accredited forensic DNA lab that also manufactures and sells products worldwide.

AXOScience

Booth Number: B-03
Company/Institute Name AXO Science – STK Sperm Tracker
Address 66 BD NIELS BOHR CEI 1 69100 VILLEURBANNE FRANCE
Website www.axoscience.com/stk
Tel +33 4 78 93 08 26

After years of research in collaboration with French National Scientific Police, AXO Science is now bringing to the forensic experts STK Sperm Tracker that improves both sensitivity and specificity of semen detection.

STK Sperm Tracker is based on an innovative technology and a new way to immobilize molecule. It offers many advantages to forensic laboratories personnel in detecting semen stains:
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- Specific: no false positive, only semen will show
- Easy: simple 3 steps protocol (spray water on STK, press 3′, read result)
- Safe: unlike regular AP Spot tests STK does NOT use any carcinogen reagents!

ademtech

Booth Number: B-04
Company/Institute Name ADEMTECH
Address 27 Allée Charles Darwin, Bat C Etage 1, 33 600 PESSAC – France
Website www.ademtech.com

Tel +33 557 020 200

ADEMTECH is a French manufacturer of DNA extraction solutions based on its own magnetic particles technologies. We provide with solutions for DNA normalization for references samples and DNA extraction for casework samples. The unique properties of our magnetic particles allow performing consistent DNA capture and ensuring to recover DNA even from very low DNA input samples or contaminated with inhibitors.
We provide ready to use solutions from manual use to very high throughput and assistance for the set up. For medium throughput ADEMTECH has designed two DNA extraction instruments in order to process from 1 to 24 samples.

ANDE

Booth Number: B-05
Company/Institute Name ANDE
Address 266 Second Avenue, Waltham, MA 02451
Website www.ande.com
Tel (781) 916-8301

ANDE is on a mission to create a safer world through Rapid DNA technology.

DNA is the gold standard for identification. However, it can take weeks or months to get results. ANDE can get a DNA identification in less than two hours and allow law enforcement to get the right answers, right away.

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By reducing crime, exonerating the innocent, catching perpetrators of sexual assault, and reuniting families; ANDE is carrying out its mission to make the world safer, one DNA identification at a time.

BIONEER

Innovation • Value • Discovery

Booth Number: B-06
Company/Institute Name Bioneer Corporation
Address 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
Website www.bioneer.com
Tel +82-42-930-8777 (Korea: 1588-9788)

Bioneer was founded in 1992 as an R&D oriented biotechnology company in South Korea. As a vertically integrated corporation, Bioneer has core competencies in oligonucleotide based therapeutics using proprietary DNA/RNA synthesis chemistry, novel molecular biology enzymes and reagents, and instrumentation. Its innovative culture enables the company to provide comprehensive solutions to academia and the molecular diagnostic industry, while the novel technologies it develops become strong foundations for new drug development. The company has wholly owned subsidiaries in the U.S. and China. Bioneer (064550.KQ) is a public company being traded on KOSDAQ, a South Korea stock exchange market.

Booth Number: B-07

Company/Institute Name	LGC - ParaDNA
Address	LGC, Queens Road, Teddington, Middlesex, TW11 0LY, UK
Website	lgcgroup.com/paradna
Tel	+44 (0) 844 2641 999

The LGC ParaDNA® System is already being used around the globe to support sample prioritisation and criminal intelligence gathering, using DNA profile information that is generated in 75 minutes. Now, a body fluid identification test with the capacity to detect single-source or mixtures of vaginal fluid, seminal fluid, sperm, saliva, blood and menstrual blood has been launched. Results can be reviewed immediately using onboard software, which also offers compare and search options for captured data or imported databases. Identify or exclude individuals/missing persons, or build forensic casework data to support victim statements, whilst reducing time/costs associated with processing forensic evidence.

Booth Number: B-08

Company/Institute Name	Douzone Bizon Co., Ltd
Address	Namsan-myeon, 130, Beodeul 1-gil, Chunchent-si, Gangwon-do, Korea
Website	http://www.douzone.com http://dforensic.com
Tel	+82 6233 2075

Douzone Bizon is an ICT corporation representing South Korea that provides ICT solutions and services tailored to companies' business needs. It was founded in 1991.
The company not only provides ERP & Groupware, electronic tax solutions but also digital forensic solution development, system establishment & consulting, forensic training and services for analysis. Additionally, Douzone has positioned itself as an ICT security consulting firm such as establishing national digital forensic laboratories for various government agencies worldwide.

▪ Digital Forensic Center (D-Forensic Center)

Douzone Forensic Center (D-Forensic Center) was founded by merging Douzone Security Center and D-Cloud IDC and recruiting digital forensic experts.
There are mainly 4 business areas which consist of digital forensic solution developments, solution distributions, consulting services and forensic training.

Booth Number: B-09, B-10

Company/Institute Name	COPAN
Address	Via F.Perotti 18,25125 Brescia, Italy
Website	www.copangroup.com
Tel	+39 030-3666367

With a rightful reputation for innovation, COPAN is the world leading manufacturer of collection and transport systems. In like manner, COPAN's collaborative approach to pre-analytics has resulted in the

development of FLOQSwabs®, ESwab®, UTM® and modular laboratory automation, WASP® and WASPLab®. For further information on COPAN, visit www.copangroup.com.

Booth Number: B-11
Company/Institute Name Hamilton Robotics
Address 4970 Energy Way, Reno, NV 89502
Website hamiltoncompany.com/robotics
Tel 800-648-5950

Hamilton Robotics is a global leader in the manufacture of automated pipetting workstations. STAR and NIMBUS product lines employ innovative liquid handling technologies and have been successfully tested with leading commercial DNA extraction and PCR setup kits, following validation criteria in accordance with SWGDAM guidelines and FBI QA Standards.

Booth Number: B-12
Company/Institute Name HEALTH Gene Technologies Co.,Ltd.
Address 396 Mingzhu Road, Hi-Tech Zone, Ningbo, P.R.China 315040
Website www.healthgenetech.net
Tel +86-574-27978799

HEALTH Gene Technologies Co.,Ltd. (HGT) is committed to developing and manufacturing simple, rapid and effective nucleic acid (DNA) testing solutions for molecular diagnostics, clinical research and forensic analysis.
HGT's Forensic Science products under the brand names of STRtyper and SureID products address the increasing needs of the forensic community.

Booth Number: B-13
Company/Institute Name KOGENE BIOTECH
Address RM1101, C-dong, Gasan digital 1-ro, Geumcheon-gu, Seoul, Korea
Website www.kogene.co.kr
Tel 82-2-2026-2150

Since KogeneBiotech was founded in 2000 our company has earned a reputation for innovation in Molecular Diagnostics. Our main business units are the production of Real-time PCR/conventional PCR Kit based on the active R&D works and Testing Service for Food and Human.
We have put major efforts in searching a new market, and new R&D works of the genetic analysis, so we have been successful at expanding the base of Real-time PCR technique in clinical diagnostics area. As a result, KogeneBiotech is the Korean industry leader in the field of PCR/Real-time PCR Kit market for Human Molecular Diagnostics and Food Genetic Analysis.

Booth Number: B-14, B-15

Company/Institute Name Illumina
Address PMAA Jaram Building 16th Floor, 78, Mapo-daero, Mapo-gu, Seoul 04168, Korea
Website www.illumina.com
Tel
Focused Forensic Power from Illumina

One Solution for all: forensic DNA testing for criminal casework, use of identity and ancestry markers as predictive DNA to provide intelligence information to ongoing investigations, forensic profiling for DNA databases, mtDNA analysis to identify missing persons and disaster victim identification.

More conclusive results in forensic testing are now a practical reality thanks to advances in genomics. Illumina's Massively Parallel Sequencing (MPS) technology and informatics deliver far more insight from forensic DNA than traditional methods, with a much higher resolution than ever before possible. You'll find the answers you need efficiently with a greater degree of certainty, with the focused power of the MiSeq FGx Forensic Genomics System.

Seek the truth with Illumina Forensic Genomics technology.

Booth Number: B-16
Company/Institute Name ISFG 2019 PRAGUE, CZECH REPUBLIC
Address Czech-In s.r.o., 5. kvetna 65, 140 21, Prague 4, Czech Republic
Website ttp://www.isfg2019.org/ , www.c-in.eu
Tel +420 727 803 220
ISFG 2019:
The 28th ISFG Congress will be held in Prague, Czech Republic, September 9-14, 2019 at the Prague Congress Centre which is one of the most dominant landmarks of the capital with wonderful panoramic views of Prague. Due to its central location, the Prague Congress Centre is easily accessible from all hotels within the city. Further to this the congress centre has a strategic advantage with over 800 hotel rooms available within less than 5 minutes walking distance. With all of the key requirements within 500 metres walking distance and just 5 minutes to the city centre via complimentary public transport provided, Prague provides a fantastically convenient solution for all ISFG Congress delegates.
Priorities: bring both the best scientists from the field of forensic genetics from all over the world and a maximum number of PhD students and early postdoctoral fellows both from European and non-European countries. The congress would be an excellent opportunity for forming new regional collaborations and networks, as well as providing a format for further introducing international scientists to their central and eastern European colleagues.

Congress Secretariat C-IN:
With over 14 years of experience, C-IN provides the highest level of services specialising in congress and association management, corporate events and special event production. Since 2013, C-IN is the only member of International Association of Professional Congress Organisers (IAPCO) with headquarters situated in the Czech Republic. Our team of 55 people delivers more than 50 events per year and acts as a long term partner for several European associations. Knowledge and experience in anticipating the meeting industry dynamics, stakeholders' expectations and entrepreneurial drive are our key values to our customers.

검찰
PROSECUTION SERVICE

Booth Number: B-17

Company/Institute Name	Supreme Prosecutors' Office
Address	157, Banpo-daero, Seochodong, Seoul, Korea
Website	www.spo.go.kr
Tel	82-2-3480-2337

The Presecution Service is the leading law enforcement authority in the Republic of Korea. Our mission is to maintain a rule of law, and provide a safe nation for the people. It is our duty to keep every individual and their loved ones safe and secure. To this end, we investigate crimes, supervise law enforcement officers, initiate and prosecute criminal case in court, and enforce court orders. We help build a free and democratic society based on principles of law. The Supreme Prosecutors' Office(SPO) oversees all local prosecution officers and runs a Forensic Science Investigation Department which assists investigation fast and accurate analysis on criminal evidence and systemic response to digital forensic and cybercrimes.

biotechnology
KOMABIOTECH

Booth Number: B-18

Company/Institute Name	KOMA BIOTECH
Address	19F, IS BIZ Tower, Yangpyeong-ro 21 gil 26, Yeongdeungpo-gu, Seoul 07207, Korea
Website	www.komabiotech.com
Tel	82-2-2660-5660

KOMA BIOTECH, based in Seoul, Korea, manufactures and provides reagents and tools for life science research. KOMA also dedicates to Forensic DNA application for human DNA identification and provides kits that amplify STR loci from the forensic samples including blood, saliva, buccal swab and saliva/blood-spotted FTA Cards. KOMA provides conventional DNA-based methods as well as direct amplification method which has no need for DNA purification. Kplex-15, Kplex-23 and Kplex-Y17 (male-specific STR genotyping) are available.

GMBC GreenMate
Biotech Corp.

Booth Number: B-19

Company/Institute Name	Greenmate Biotech Corp
Address	149(Gasan-dong, Shinhan Innoplex), Gasan digital 1-ro, Geumcheon-gu, Seoul, Korea
Website	www.greenmate.co.kr
Tel	82-2-581-0131

Greenmate Biotech Company was established to become the foundation of domestic life science development.
Through its partnership with the Global Science and Technology Company, Greenmate Biotech Corp. offers the best research equipment and skills needed for life sciences research. In the forensic, we

provide automated equipment for gene library and Dried Blood Spot equipment.
Besides, we have a leading position in Multimode Microplate reader, Thermocycler, Nano volume photometer.

Booth Number: B-20
Company/Institute Name Bio-Medical Science Co., Ltd.
Address 22 Yeoksam-ro 7-gil, Gangnam-gu, Seoul, KOREA, 06244
Website www.bmskorea.co.kr
Tel +82-2-3471-6500

Working with numerous reputable global companies since 1988, Bio-Medical Science Co., Ltd. handles and distributes basic to the most advanced laboratory products, and provides quality services to satisfy a variety of demands from the field of life sciences such as molecular biology and genetics.
Headquartered in the heart of Seoul, BMS primarily serves the government, biotechnology, life science, education, and clinical sectors. With its newly constructed distribution center near Gimpo International Airport, and strategically placed sales office in Deajeon to cover the rest of the southern part of Korea, BMS is known for its highest professional standards and industry leading credentials.

Booth Number: B-21
Company/Institute Name Miltenyi Biotec
Address Friedrich-Ebert-Strasse 68
Website www.miltenyibiotec.com
Tel 0049220483060

Miltenyi Biotec is a global provider of products and services that advance biomedical research and cellular therapy. Our innovative tools support research at every level, from basic research to translational research to clinical application. Used by scientists and clinicians around the world, our technologies cover techniques of sample preparation, cell isolation, cell sorting, flow cytometry, and cell culture. Our 25 years of expertise spans research areas including immunology, stem cell biology, neuroscience, and cancer. Today, Miltenyi Biotec has more than 1,800 employees in 25 countries – all dedicated to helping researchers and clinicians make a greater impact on science and health.

Booth Number: B-22
Company/Institute Name Elsevier Forensics
Address Elsevier, The Boulevard, Kidlington, Oxford OX5 1GB, United Kingdom
Website www.elsevier.com
Tel +441865843907

Elsevier provides information and analytics that help institutions and professionals progress science, advance healthcare and improve performance. Elsevier publishes over 2,500 digitized journals, more than 35,000 book titles and many iconic reference works.
The Elsevier Forensics journals portfolio includes titles like Forensic Science International, Science & Justice, Legal Medicine and the ISFG journal, Forensic Science International: Genetics.

Booth Number: B-23

Company/Institute Name	qualitype GmbH
Address	Moritzburger Weg 67 / 01109 Dresden / Germany
Website	www.qualitype.de
Tel	+49 351 8838 2800

The qualitype GmbH is a software system supplier for digital laboratory and manufacturing processes based in Germany. Due to the digital qualification of raw data including their evaluation we support the clarification of paternity- and complex kinship questions as well as the analysis of crime scene samples since 2001. Our solutions for analytic questions in the field of DNA and genetics are GenoProof, GenoProof Mixture and Genolab. All systems can be used immediately and without adjustments in your laboratory. Come and visit us at booth B-23!

Booth Number: B-24

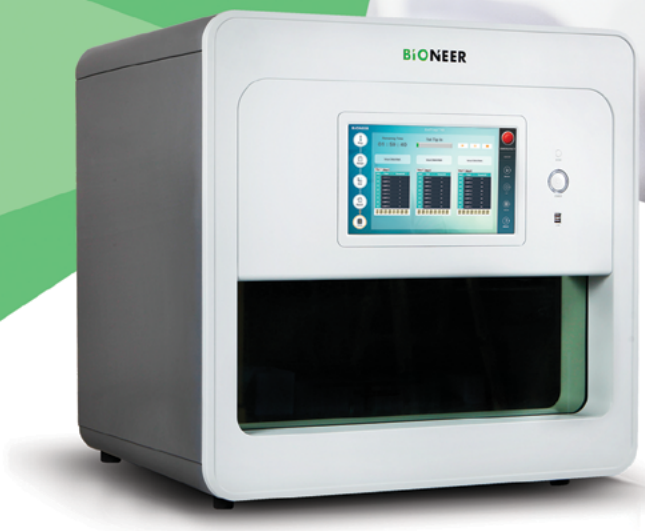
Company/Institute Name	STRmix™
Address	Institute of Environmental Science and Research Limited, Private Bag 50348, Porirua 5240, New Zealand
Website	http://strmix.esr.cri.nz
Tel	+64 4 914 0681

STRmix™ is expert forensic software that can resolve previously unresolvable complex DNA mixtures. It uses a world leading, fully continuous approach for DNA profile interpretation. Using standard, well-established statistical methods, STRmix™ builds up a picture of the DNA genotypes that, when added together, best explain the observed mixed DNA profile. STRmix™ then enables users to compare the results against a person or persons of interest and calculate a statistic, or “likelihood ratio,” of the strength of the match. Please come and see us at our booth for a demonstration of our latest release, STRmix™ v2.5.

ExiPrep™ 48

Fully Automated Nucleic Acid Extraction System

- Extract nucleic acids from up to 48 different samples per run
- High-yield, high-purity nucleic acids using Bioneer's magnetic nanobead technology
- Increased productivity, reproducibility, and reliability by full automation incl. pipetting and punching



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본　　사 : 대전광역시 대덕구 문평서로 8-11 (문평동)　www.bioneer.co.kr
바이오니아R&D센터 : 경기도 성남시 분당구 대왕판교로 700(삼평동) 코리아바이오파크 B동 702호

ISFG 2017 SEOUL

Program

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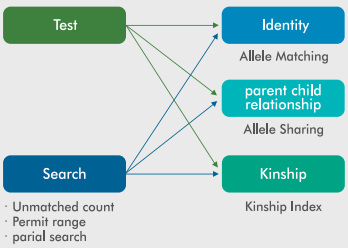
"Very fast approach Human DNA I.D. and Kinship analysis"

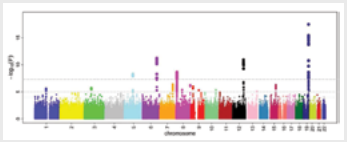
- Identity, Paternity, Kinship test and search using A-STR, Y-STR, mtDNA, SNP
- Fast Approximate Search Algorithm for matching and sharing
- Combination of various genotypes
- Partial search for genotypes and alleles
- Allele frequency table management



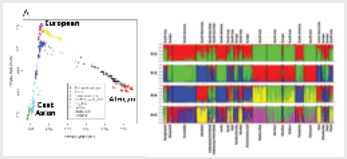
incoGWAS™

"Easy to use genome-wide association study"

- Identity, Paternity, Kinship test and search using A-STR, Y-STR, mtDNA, SNP
- Fast Approximate Search Algorithm for matching and sharing
- Combination of various genotypes
- Partial search for genotypes and alleles
- Allele frequency table management



General visualization of GWAS - Manhattan plot

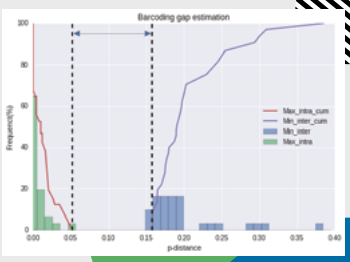


Examples of population analysis by PCA and structure

incoBARCODE ID™

"Accurate non-human species I.D. using DNA barcode"

- Identity, Paternity, Kinship test and search using A-STR, Y-STR, mtDNA, SNP
- Fast Approximate Search Algorithm for matching and sharing
- Combination of various genotypes
- Partial search for genotypes and alleles
- Allele frequency table management



INSILICOGEN
www.insilicogen.com
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+82.31.278.0061
www.insilicogen.com

PROGRAM

August 29 (Tue), 2017

18:30 - 19:30 **ISFG Scientific Prize Lecture (Auditorium, 3F, Coex)**

Thomas Parsons
- Forensic genetics and the missing: Striving for certainty and justice

19:30 - 20:00 **Opening Ceremony (Auditorium, 3F, Coex)**

20:00 -21:30 **Welcome Reception (Grand Ballroom 103+104, 1F, Coex)**

August 30 (Wed), 2017

Session 1 Massively Parallel Sequencing

Chairs: Walther Parson, HwanYoung Lee

09:00 - 09:45 **Keynote 1 (Auditorium, 3F, Coex)**

Kyoung-Jin Shin
Implementing massively parallel sequencing for forensic DNA analysis using in-house PCR panels

09:45 - 13:00 **Oral Session (Auditorium, 3F, Coex)**

09:45 - 10:00 **Laurence Alicia Elisabeth Devesse**
The Tao of MPS: Common novel variants

10:00 - 10:15 **Christopher Phillips**
A missing persons identification panel for the MPS era

10:15 - 10:30 **Bruce Budowle**
Massively parallel sequencing (MPS) can be considered NGS, i.e., now generation sequencing: Implementation of whole genome mitochondrial DNA sequencing into routine casework

10:30 - 11:30 **Coffee Break (Auditorium Lobby, 3F, Coex)**
& Poster Session (Grand Ballroom 101+102, 1F, Coex)

11:30 - 11:45 **Katherine Butler Gettings**
STRseq: A resource for sequence-based STR analysis

11:45 - 12:00 **Sacha Willuweit**
NOMAUT - NGS STR nomenclature for forensic genetics

12:00 - 12:15 **Sebastian Ganschow**
ToaSTR: A web-based forensic tool for the analysis of short tandem repeats in massively parallel sequencing data

12:15 - 12:30 **SallyAnn Harbison**
Establishing the limit of detection of massively parallel sequencing using laser micro-dissected cells

12:30 - 12:45 **Chiara Turchi**
A microhaplotypes panel for forensic genetics using massive parallel sequencing (MPS)

12:45 - 13:00 **Jennifer Churchill**
Massively parallel sequencing-enabled mixture analysis of mitochondrial DNA samples

13:00 - 14:30 **Lunch Seminar hosted by Thermo Fisher Scientific**

13:00 - 13:40 **Seminar (Auditorium, 3F, Coex)**

13:40 - 14:30 **Lunch (Grand Ballroom 103+104, 1F, Coex)**

Session 2 Population Genetics, Lineage Markers, Ancestry, Paternity

Chairs: Leonor Gusmao, Lutz Roewer

14:30 - 15:15 **Keynote 2 (Auditorium, 3F, Coex)**

Mark Jobling
Genetic and genomic approaches to estimating ancestry

15:15 - 18:15 **Oral Session (Auditorium, 3F, Coex)**

15:15 - 15:30 **Michael Nothnagel**
Revisiting the male genetic landscape of China

15:30 - 15:45 **Torben Tvedebrink**
GenoGeographer - A tool for genogeographic inference

15:45 - 16:00 **Martin Bodner**
Discerning the "identical": Extended mitogenome diversity behind the most common West Eurasian mtDNA control region haplotype

16:00 - 17:00 **Coffee Break (Auditorium Lobby, 3F, Coex)**
& Poster Session (Grand Ballroom 101+102, 1F, Coex)

17:00 - 17:15 **Anu Maria Neuvonen**
The effect of a singular population structure on forensic analysis

17:15 - 17:30 **YunGeon Kim**
Development of ethnic classification models for Asian group: A data mining approach

17:30 - 17:45 **Runa Daniel**
An ancestry-informative assay for the Asia Pacific region

17:45 - 18:00 **Nor Aidora binti Saedon**
The applications of Malaysian populations plotted via next generation sequencing

18:00 - 18:15 **Arwin Ralf**
Increasing the number of Rapidly Mutating (RM) Y-STRs significantly improves male relative differentiation

18:30 - 19:30 **Working Group Meeting**

August 31 (Thu), 2017

Session 3 Non-Human, Microbiome, extra MPS

Chairs: Mechthild Prinz, Chang-Bae Kim

09:00 - 09:45 **Keynote 3 (Auditorium, 3F, Coex)**

Mehrdad Hajibabaei
Genomics approaches for characterizing biological diversity : From identifying species to monitoring whole ecosystems

09:45 - 13:00 **Oral Session (Auditorium, 3F, Coex)**

09:45 - 10:00 **Adrian Linacre**
'ForCyt' DNA Database of wildlife species

10:00 - 10:15 **Sungmin Kim**
Non-human forensic identification of complex mixture samples using DNA metabarcoding

10:15 - 10:30 **Burkhard Berger**
Canine STR marker get more - Dog breed affiliation with a forensically validated STR panel

10:30 - 11:30 **Coffee Break (Auditorium Lobby, 3F, Coex)**
& Poster Session (Grand Ballroom 101+102, 1F, Coex)

11:30 - 11:45 **Sorelle Bowman**
Identification of hoax agents, Bacillus and Yersinia strains by protein profiling using microfluidic capillary electrophoresis with peak detection algorithms

11:45 - 12:00 **Natasha Arora**
Micro biome communities analyses to predict body sites: Analyses on published data and on exposed samples in the laboratory

12:00 - 12:15 **Weibo Liang**
Microhaplotype: Ability of persanal idenfication and being ancestry informative marker

12:15 - 12:30 **Daniele Podini**
Microhaplotype for ancestry prediction

12:30 - 12:45 **Ji Hyun Lee**
Prediction of Asian ethnic subgroups using HID-Ion AmpliSeqTM Ancestry Panel

12:45 - 13:00 **Wibhu Kutanan**
Complete mitochondrial genomes of the hunter-gatherer populations in Thailand

13:00 - 14:30 **Lunch Seminar hosted by Promega Corporation**

13:00 - 13:40 **Seminar (Auditorium, 3F, Coex)**

13:40 - 14:30 **Lunch (Grand Ballroom 103+104, 1F, Coex)**

Session 4 Body Fluid, Touch DNA, Forensic Biology

Chairs: Peter Schneider, Yiping Hou

14:30 - 15:15 **Keynote 4 (Auditorium, 3F, Coex)**

Titia Sijen
DNA transfer and cell type inference to assist activity level reporting

15:15 - 18:15 **Oral Session (Auditorium, 3F, Coex)**

15:15 - 15:30 **Sabrina Ingold**
Association of a body fluid with a DNA profile by targeted RNA/DNA deep sequencing

15:30 - 15:45 **Phyvadol Thanakiatkrai**
Direct PCR improves STR profiles from substrates of improvised explosive device

15:45 - 16:00 **Lode Sibbens**
The development of a forensic clock to determine time of death

16:00 - 17:00 **Coffee Break (Auditorium Lobby, 3F, Coex)**
& Poster Session (Grand Ballroom 101+102, 1F, Coex)

17:00 - 17:15 **Alicia Haines**
Latent DNA detection using fluorescent DNA binding dyes

17:15 - 17:30 **Bianca Szkuta**
Assessment of the prevalence, transfer, persistence and recovery of DNA traces from worn upper garments: Results from an inter-laboratory study

17:30 - 17:45 **Qingzhen Meng**
DNA transfer study based on parallel system methods

17:45 - 18:00 **Athina Vidaki**
CpGforID: Epigenetic discrimination of identical twins under the forensic scenario

18:00 - 18:15 **Diana H Hall**
Application of DIP-STRs to sexual/physical assault investigations: Eight case reports

18:30 - 19:30 **General Assembly of the ISFG (Auditorium, 3F, Coex)**

September 1 (Fri), 2017

Session 5 Predictive Markers, Biostatistics

Chairs: John Butler, Titia Sijen

09:00 - 09:45 **Keynote 5 (Auditorium, 3F, Coex)**

Manfred Kayser
Advances in human appearance genetics and relevance for forensic DNA phenotyping

09:45 - 13:00 **Oral Session (Auditorium, 3F, Coex)**

09:45 - 10:00 **Sae Rom Hong**
Epigenetic age signatures in saliva: Age prediction using methylation SNaPshot and massive parallel sequencing

10:00 - 10:15 **Ewelina Pospiech**
Epigenetic prediction of age in different study groups

10:15 - 10:30 **Lin Zhang**
Association between BMP4 gene polymorphisms and eyelid traits in Chinese Han population

10:30 - 11:30 **Coffee Break (Auditorium Lobby, 3F, Coex)**
& Poster Session (Grand Ballroom 101+102, 1F, Coex)

11:30 - 11:45 **Chie Morimoto**
Discriminating between uncle-nephew and grandfather-grandson kinships by analyzing chromosomal sharing patterns

11:45 - 12:00 **James Curran**
A new (approximate) method for Y-STR haplotype probability assignment

12:00 - 12:15 **Mikkel Meyer Andersen**
How convincing is a matching Y-chromosome profile?

12:15 -12:30 **Øyvind Bleka**
Using EuroForMix to analyse complex SNP mixtures, up to six contributors

12:30 - 12:45 **Catherine Elizabeth McGovern**
Establishing a range of foundational validity for complex DNA mixture interpretation using probabilistic genotyping software: A response to PCAST

12:45 - 13:00 **Charles Hallam Brenner**
Impossible to specify the number of contributors to a DNA mixture

13:00 - 14:30 **Lunch Seminar hosted by Menarini Silicon Biosystem**

13:00 - 13:40 **Seminar (Auditorium, 3F, Coex)**

13:40 - 14:30 **Lunch (Grand Ballroom 103+104, 1F, Coex)**

Session 6 Ethics & Legal, DNA Databases, DVI

Chairs: Denise Syndercomb-court, Seung Hwan Lee

14:30 - 15:15 **Keynote 6 (Auditorium, 3F, Coex)**

Susan Friedman
Science advancing justice : Lessons learned from DNA exonerations

15:15 - 17:00 **Oral Session (Auditorium, 3F, Coex)**

15:15 - 15:30 ※ *The presentation is cancelled. Alternative speaker will be determined.*

15:30 - 15:45 ※ *The presentation is cancelled. Alternative speaker will be determined.*

15:45 - 16:00 **Marta Diepenbroek**
First genetic evidence for Jewish holocaust victims in Sobibor, Poland, by phylogeographic analyses

16:00 - 16:15 **Coffee Break (Auditorium Lobby, 3F, Coex)**

16:15 - 16:30 ※ *The presentation is cancelled.*

16:30 - 16:45 **Maria Geppert**
Wider use of DNA evidence in criminal cases: Ancestry prediction using Y chromosome analysis in Germany

16:45 - 17:00 **Kyleen Elwick**
Comparative tolerance of two massively parallel sequencing platforms to common PCR inhibitors for missing person cases

18:30 - 22:00 **Gala Dinner & After Party (Grand Walkerhill Seoul)**

September 2 (Sat), 2017

Session 7 New Technologies, DNA Typing Methods, Quality Control

Chairs: SallyAnn Harbison, Byungwon Chun

09:00 - 11:15 **Oral Session (Auditorium, 3F, Coex)**

09:00 - 09:15 **Patrick Basset**
Lessons from a study of DNA contaminations from police services and forensic laboratories in Switzerland

09:15 - 09:30 **MARTIN EDUARDO MAUTNER**
Degraded DNA samples made informative by using superprimers

09:30 - 09:45 **Suni Edson**
DNA typing from skeletal remains: A study of inhibitors using mass spectrometry

09:45 - 10:00 **Fabio Macêdo Mendes**
Kpop: A Python package for population genetics analysis

10:00 - 10:15 **Coffee Break (Auditorium Lobby, 3F, Coex)**

10:15 - 10:30 **David John Moore**
A large scale study on the characterisation of drop-in within a DNA laboratory

10:30 - 10:45 **Lei Feng**
Protein-based forensic identification using hair shaft in east Asian and Eurasian populations

10:45 - 11:00 **Becky Steffen**
Beyond the STRs: A comprehensive view of current forensic DNA markers characterized in the PCR-Based DNA Profiling Standard (SRM 2391D)

11:00 - 11:15 **Michelle Peck**
Inter-laboratory validation study of the ForenSeq DNA Signature Prep Kit, MiSeq FGx instrument, and ForenSeq universal analysis software for length-based STR analysis

11:15 - 12:00 **Closing Ceremony (Auditorium, 3F, Coex)**

ISFG 2017 SEOUL

Oral Abstracts

ORAL ABSTRACTS

No.	Presenting Author	Title
1	Thomas Parsons	Forensic genetics and the missing: Striving for certainty and justice
2	Kyoung-Jin Shin	Implementing massively parallel sequencing for forensic DNA analysis using in-house PCR panels
3	Laurence Alicia Elisabeth Devesse	The Tao of MPS: Common novel variants
4	Christopher Phillips	A missing persons identification panel for the MPS era
5	Bruce Budowle	Massively parallel sequencing (MPS) can be considered NGS, i.e., now generation sequencing: Implementation of whole genome mitochondrial DNA sequencing into routine casework
6	Katherine Butler Gettings	STRseq: A resource for sequence-based STR analysis
7	Sascha Willuweit	NOMAUT - NGS STR nomenclature for forensic genetics
8	Sebastian Ganschow	ToaSTR: A web-based forensic tool for the analysis of short tandem repeats in massively parallel sequencing data
9	SallyAnn Harbison	Establishing the limit of detection of massively parallel sequencing using laser micro-dissected cells
10	Chiara Turchi	A microhaplotypes panel for forensic genetics using massive parallel sequencing (MPS)
11	Jennifer Churchill	Massively parallel sequencing-enabled mixture analysis of mitochondrial DNA samples
12	Mark Jobling	Genetic and genomic approaches to estimating ancestry
13	Michael Nothnagel	Revisiting the male genetic landscape of China
14	Torben Tvedebrink	GenoGeographer - A tool for genogeographic inference
15	Martin Bodner	Discerning the "identical": Extended mitogenome diversity behind the most common West Eurasian mtDNA control region haplotype
16	Anu Maria Neuvonen	The effect of a singular population structure on forensic analysis
17	YunGeon Kim	Development of ethnic classification models for Asian group: A data mining approach
18	Runa Daniel	An ancestry-informative assay for the Asia Pacific region
19	Ji hyun Lee	Prediction of Asian ethnic subgroups using HID-Ion AmpliSeq™ Ancestry Panel
20	Arwin Ralf	Increasing the number of Rapidly Mutating (RM) Y-STRs significantly improves male relative differentiation
21	Mehrdad Hajibabaei	Genomics approaches for characterizing biological diversity : From identifying species to monitoring whole ecosystems
22	Adrian Linacre	'ForCyt' DNA Database of wildlife species

23	Sungmin Kim	Non-human forensic identification of complex mixture samples using DNA metabarcoding
24	Burkhard Berger	Canine STR marker get more - Dog breed affiliation with a forensically validated STR panel
25	Sorelle Bowman	Identification of hoax agents, *Bacillus* and *Yersinia* strains by protein profiling using microfluidic capillary electrophoresis with peak detection algorithms
26	Natasha Arora	Microbiome communities analyses to predict body sites: Analyses on published data and on exposed samples in the laboratory
27	Weibo Liang	Microhaplotype: Ability of persanal idenfication and being ancestry informative marker
28	Daniele Podini	Microhaplotype for ancestry prediction
29	Nor Aidora binti Saedon	The applications of Malaysian populations plotted via next generation sequencing
30	Wibhu Kutanan	Complete mitochondrial genomes of the hunter-gatherer populations in Thailand
31	Titia Sijen	DNA transfer and cell type inference to assist activity level reporting
32	Sabrina Ingold	Association of a body fluid with a DNA profile by targeted RNA/DNA deep sequencing
33	Phuvadol Thanakiatkrai	Direct PCR improves STR profiles from substrates of improvised explosive device
34	Lode Sibbens	The development of a forensic clock to determine time of death
35	Alicia Haines	Latent DNA detection using fluorescent DNA binding dyes
36	Bianca Szkuta	Assessment of the prevalence, transfer, persistence and recovery of DNA traces from worn upper garments: Results from an inter-laboratory study
37	Qingzhen Meng	DNA transfer study based on parallel system methods
38	Athina Vidaki	CpGforID: Epigenetic discrimination of identical twins under the forensic scenario
39	Diana H Hall	Application of DIP-STRs to sexual/physical assault investigations: Eight case reports
40	Manfred Kayser	Advances in human appearance genetics and relevance for forensic DNA phenotyping
41	Sae Rom Hong	Epigenetic age signatures in saliva: Age prediction using methylation SNaPshot and massive parallel sequencing
42	Ewelina Pośpiech	Epigenetic prediction of age in different study groups
43	Lin Zhang	Association between BMP4 gene polymorphisms and eyelid traits in Chinese Han population
44	Chie Morimoto	Discriminating between uncle-nephew and grandfather-grandson kinships by analyzing chromosomal sharing patterns

45	James Curran	A new (approximate) method for Y-STR haplotype probability assignment
46	Mikkel Meyer Andersen	How convincing is a matching Y-chromosome profile?
47	Øyvind Bleka	Using EuroForMix to analyse complex SNP mixtures, up to six contributors
48	Catherine Elizabeth McGovern	Establishing a range of foundational validity for complex DNA mixture interpretation using probabilistic genotyping software: A response to PCAST
49	Charles Hallam Brenner	Impossible to specify the number of contributors to a DNA mixture
50	Susan Friedman	Science advancing justice : Lessons learned from DNA exonerations
53	Marta Diepenbroek	First genetic evidence for Jewish holocaust victims in Sobibor, Poland, by phylogeographic analyses
54	Turi King	Who's the daddy? Tracing the break in the Y chromosome lineage of King Richard III
55	Maria Geppert	Wider use of DNA evidence in criminal cases: Ancestry prediction using Y chromosome analysis in Germany
56	Kyleen Elwick	Comparative tolerance of two massively parallel sequencing platforms to common PCR inhibitors for missing person cases
57	Patrick Basset	Lessons from a study of DNA contaminations from police services and forensic laboratories in Switzerland
58	MARTIN EDUARDO MAUTNER	Degraded DNA samples made informative by using superprimers
59	Suni Edson	DNA typing from skeletal remains: A study of inhibitors using mass spectrometry
60	Fabio Macêdo Mendes	Kpop: A Python package for population genetics analysis
61	David John Moore	A large scale study on the characterisation of drop-in within a DNA laboratory
62	Lei Feng	Protein-based forensic identification using hair shaft in east Asian and Eurasian populations
63	Becky Steffen	Beyond the STRs: A comprehensive view of current forensic DNA markers characterized in the PCR-Based DNA Profiling Standard (SRM 2391D)
64	Michelle Peck	Inter-laboratory validation study of the ForenSeq DNA Signature Prep Kit, MiSeq FGx instrument, and ForenSeq universal analysis software for length-based STR analysis

ISFG 2017 SEOUL

1. FORENSIC GENETICS AND THE MISSING: STRIVING FOR CERTAINTY AND JUSTICE

ISFG Scientific Prize Lecture

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In it's magnitude of importance and range of applications, forensic genetics has profoundly influenced society. One area of greatest impact is the identification of missing persons. When loved ones go missing, humans have a fundamental need for knowledge of their fate and the return of mortal remains for commemoration. Society demands effective state and institutional mechanisms for accounting for the missing, including provision for justice. Forensic genetics must therefore accommodate many contexts: DVI from accidents, natural disaster or terrorism; military missing in action; mass graves and war crimes; deaths due to migration; "routine" missing persons; human trafficking; and re-uniting living persons who have been separated from families.
This lecture will review the development and application of DNA methods that have now given rise to unprecedented abilities to address the personal and societal trauma associated with missing persons. Missing persons DNA applications have drawn from developments in other forensic applications, but they have also been a driving force in the development and refinement of methods in many genetic systems. This includes not only DNA marker systems, but informatics and interpretation frameworks, for example in kinship analysis or rigorous multi-disciplinary integration of evidence. Massively Parallel Sequencing is beginning to be implemented, and will offer powerful new capabilities. Our field must ensure that optimal systems are developed for the missing, and to promote as well the establishment of policies and collaborative mechanisms that will permit technical advances to be brought to bear on this area of critical importance to humanity and human rights.

2. IMPLEMENTING MASSIVELY PARALLEL SEQUENCING FOR FORENSIC DNA ANALYSIS USING IN-HOUSE PCR PANELS

Keynote Speech

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Massively parallel sequencing (MPS) has been increasingly used for the analysis of various forensic markers to complement the weak points of capillary electrophoresis and to obtain more data while using only one platform. However, there are several obstacles in the current research and practical application of forensic DNA analysis using the MPS method. The first is the lack of feedback for further progress due to limited public information of available commercial MPS panels. The second are the costly and time-consuming procedures used to prepare the MPS library. Finally, the MPS data analysis is somewhat of a hassle. Therefore we have developed in-house PCR panels which can amplify mitochondrial DNA, autosomal/Y chromosomal STRs and microhaplotypes by marker and then investigated sequence variation of these markers using the developed panels and customized MPS protocols. The cost and time spent in lab could be reduced by adopting the PCR based method instead of the adapter ligation method to prepare an MPS library with the amplicons. The generated MPS data

were analyzed using open source software; consequently, a platform independent MPS protocol was able to be established. In addition, autosomal STR genotyping for the same samples was carried out on two different MPS platforms and the results were compared. The speaker hopes that this presentation will be helpful to the researcher and/or practitioner who is using or will soon use MPS in their laboratory by discussing its application for forensic DNA analysis using in-house PCR panels and MPS protocols.

1. Børsting C1, Morling N2. Next generation sequencing and its applications in forensic genetics. Forensic Sci Int Genet. 2015;18:78-89.
2. Kim EH, Lee HY, Kwon SY, Lee EY, Yang WI, Shin KJ. Sequence-based diversity of 23 autosomal STR loci in Koreans investigated using an in-house massively parallel sequencing panel. Forensi Sci. Int. Genet. 2017; Submitted.
3. Kwon SY, Lee HY, Kim EH, Lee EY, Shin KJ. Investigation into the sequence structure of 23 Y chromosomal STR loci using massively parallel sequencing. Forensic Sci Int Genet. 2016;25:132-141.

3. THE TAO OF MPS: COMMON NOVEL VARIANTS

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The introduction of massively parallel sequencing (MPS) to forensic genetics has led to improvements in multiple aspects of DNA analysis, however additional complexities are concurrently associated with these advances. In relation to STR analysis, the move to assign alleles using sequence rather than length based methodologies has highlighted the extent to which previous allelic variation was masked. In this work, a series of samples (n=1000) from five different population groups (Caucasian, West African, North East African, East Asian and South Asian) were genotyped for 27 forensically validated autosomal STRs. Results were compared to data from the National Institute of Standards and Technology (NIST), with this collaborative project now providing one of the most expansive data sets generated using MPS technology to date. This presentation addresses the benefits and challenges associated with the discovery of novel sequence variants. The large number of these variants characterised at select markers brings into question the strategies for producing representative population data, yet also provides an opportunity to utilize this diversity in unique ways. Results from this collaborative study have demonstrated that the number of samples necessary to capture the breadth of allelic variation is highly dependent on the individual marker and the extent of its sequence variability. As one example, within a single population, all common sequence-based alleles at CSF1PO are captured when genotyping less than 200 individuals, while 400 individuals are insufficient for this purpose at D12S391.

4. A MISSING PERSONS IDENTIFICATION PANEL FOR THE MPS ERA

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We have developed a large-scale SNP-based forensic identification panel for DNA analysis with massively parallel sequencing (MPS). The panel was specifically designed for the challenges of identifying missing persons; where the DNA can be highly degraded and relationship tests may involve reference samples from across several generations and in a deficient pedigree. For this reason, the panel comprises very short DNA fragments carrying multiple-allele SNPs or small microhaplotypes - both more informative than binary SNPs. We compiled 1411 tri-allelic or tetra-allelic SNPs (1377 autosomal loci, 34 X loci) and 46 microhaplotypes into a single PCR multiplex. In the multiple-allele SNPs, only 1.5% had average Heterozygosities (averaged over five 1000 Genomes population groups) below the binary SNP maximum of 50%. Amongst the 46 microhaplotypes originally compiled by Kiddlab [1], 18 had average Heterozygosities higher than the tri-allelic SNP maximum value of 66.7%. Thirty of the microhaplotypes had their size reduced by an average of 65 nucleotides without significant loss of informativeness due to the exclusion of SNPs at the boundaries of the full haplotype. We report component marker characteristics, including 1000 Genomes population frequency estimates, and simulations of relationship testing scenarios where distant pairwise relationships need to be statistically assessed.

1. Kidd K, et al: Evaluating 130 microhaplotypes across a Global Set of 83 Populations, Forensic Sci. Int. Genet. 2017; accepted for publication.

5. MASSIVELY PARALLEL SEQUENCING (MPS) CAN BE CONSIDERED NGS, I.E., NOW GENERATION SEQUENCING: IMPLEMENTATION OF WHOLE GENOME MITOCHONDRIAL DNA SEQUENCING INTO ROUTINE CASEWORK

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Internal validation and implementation of the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific), has begun in our Missing Persons Unit. The entire genome is enriched by a 162 multiplex reaction (two amplification reactions) resulting in no amplicon greater than 175 bases in length. The mtDNA genome potentially can be sequenced from DNA typically encountered in human remains with greater resolution and no more consumption of sample than by Sanger sequencing. For MPS, library preparation and sequencing are performed on the Ion Chef and S5 (Thermo Fisher Scientific), respectively, reducing the workflow to only three pipetting steps and placing the chip into the sequencer. Since MPS technology is not yet widely used in actual casework analyses, we provide guidance on the criteria and steps to facilitate implementation. Validation entails: concordance studies, comparison of long and short PCR results, accuracy and precision (or reproducibility and repeatability), sensitivity (down to 1 pg of genomic DNA), stochastic effects (amplicon drop out), contamination, because of quantitative nature – mixture studies, degradation and inhibition, mock case samples, species specificity, and different strategies for multiplexing samples. In addition to these typical validation studies, since commercial software is not yet sufficiently robust, a pipeline of freeware and in-house

tools has been developed to process mtDNA results.
We present: internal validation studies, interpretation guidelines, thresholds and heteroplasmy, nomenclature, development of SOPs, training materials and processes for analysts, and novel workflow considerations. Our experiences could assist others considering the challenges of implementing a new platform and chemistry.

6. STRSEQ: A RESOURCE FOR SEQUENCE-BASED STR ANALYSIS

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The STR Sequencing Project was initiated to facilitate the description of sequence-based alleles at the Short Tandem Repeat (STR) loci targeted in human identification assays. This collaborative effort of the international forensic DNA community provides a framework for communication among laboratories. STRSeq data are maintained as GenBank records at the U.S. National Center for Biotechnology Information (NCBI), which participates in a daily data exchange with the DNA DataBank of Japan (DDBJ) and the European Nucleotide Archive (ENA).
Each GenBank record contains: (a) observed sequence of an STR region, (b) annotation of the repeat region ("bracketing" consistent with the guidance of the ISFG [1]) and flanking region polymorphisms, (c) information regarding the sequencing assay and data quality, and (d) backward compatible length-based allelic designation. STRSeq GenBank records are organized within a BioProject at NCBI (The STR Sequencing Project, www.ncbi.nlm.nih.gov/bioproject/380127), which is sub-divided by 1) Commonly used autosomal STR Loci, 2) Alternate autosomal STR Loci, 3) Y-chromosomal STR loci, and 4) X-chromosomal STR loci. Each of these categories is further divided into locus-specific projects. The BioProject hierarchy allows easier access to the GenBank records by browsing, BLAST searching, or ftp download; future plans include interface tools at strseq.nist.gov.
This presentation will inform attendees about the initiative, illustrate ways in which data may be accessed, and provide orientation of this project in relation to other, complementary efforts.

1. Parson W, Ballard D, Budowle B, Butler JM, Gettings KB, Gill P, Gusmão L, Hares DR, Irwin JA, King JL, Knijff Pd, Morling N, Prinz M, Schneider PM, Neste CV, Willuweit S, and Phillips C: Massively Parallel Sequencing of forensic STRs: Considerations of the DNA Commission of the International Society of Forensic Genetics (ISFG) on minimal nomenclature requirements. Forensic Science International: Genetics. 2016; 22: 54-63.

7. NOMAUT – NGS STR NOMENCLATURE FOR FORENSIC GENETICS

S. Willuweit, on behalf of the DNASeqEx EU project

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In addition to the general adoption of NGS in a forensic lab and implementation effort involved, the exchange of NGS STR results and comparison with legacy STR data is a crucial challenge when it comes to the interpretation of casework samples as part of routine work.
There are some proposals on the nomenclature of NGS STR results. Most of them are driven by the

idea of representing a (genetically) correct variant call against a reference sequence. Others try to reduce the necessary sequence information down to named repeat patterns. And finally, some are just doing in silico CE to call alleles while discarding any sequence information. Only the later does not fail to provide compatibility and comparability to the huge proportion of CE STR results already done and probably still going to be produced. Unfortunately, using this approach, we would not gain any information provided by NGS.
Since the coexistence and transition between NGS and CE is essential to forensic genetics, the NOMAUT NGS STR nomenclature incorporates compatibility and comparability. To archive this, NOMAUT was built as a huge catalogue of acquired sequence variants and the ability to grow in a very convenient but save and robust way. As a catalogue is a centralized structure, it can be imagined as an authoritative oracle answering sequence queries with allele calls - a NOMenclature AUThority. Whereas the underlying nomenclature rules and procedures are rather trivial, the accomplishment of safety and security in a worldwide scale are very challenging. To ensure for reliability and availability NOMAUT was built as a container being easily distributed over web service infrastructures like Amazons AWS.
This presentation will shed some light on the basic idea of NOMAUT as well as the initial implementation of the secure and self-maintaining authority software and the exemplary code samples provided for NGS software producers for easy adoption of NOMAUT in their software.
The intention of this presentation is to motivate scientists and representatives/providers of relevant software products to shape and support NOMAUT as a collaborative effort.

8. TOASTR: A WEB-BASED FORENSIC TOOL FOR THE ANALYSIS OF SHORT TANDEM REPEATS IN MASSIVELY PARALLEL SEQUENCING DATA

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In recent studies, massively parallel sequencing (MPS) has demonstrated its potential for the analysis of short tandem repeats (STRs) in challenging forensic casework samples. MPS can detect sequence variants in isoalleles and thus increase resolution over conventional capillary electrophoresis (CE). However, considering currently available software, data analysis turns out to be a cumbersome process especially for laboratories without bioinformatical expertise. We developed the web application toaSTR (toastr.labcon-owl.de) as a user-friendly tool for platform independent STR allele calling in MPS data. toaSTR features an intuitive graphical user interface and transparent configuration options that are continuously optimized based on the user's feedback. It enables highly customizable STR panels and gives the user the ability to select from a wide range of markers. Both commercial and in-house multiplex PCR kits can be analyzed with only minimal requirements on primer design. Due to its stutter modelling algorithm, toaSTR is able to distinguish biological alleles from stutter artefacts and assists mixture interpretation. The result report visualizes called alleles for each STR system and complies with current nomenclature recommendations for sequence alleles. Data can also be exported for further analysis in biostatistical tools. We designed an in-house multiplex STR kit to evaluate toaSTR's performance under defined conditions and to enable comparison with a commercial kit. An initial validation study with ring trial samples and reference DNA confirmed a highly reliable allele calling and full concordance with CE results. Sequence information and stutter labeling proved beneficial for the interpretation of mixed profiles.

9. ESTABLISHING THE LIMIT OF DETECTION OF MASSIVELY PARALLEL SEQUENCING USING LASER MICRO-DISSECTED CELLS

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Massively parallel sequencing is fast emerging as an increasingly useful tool for forensic science. As part of our ongoing validation of this technology we wanted to both ensure compatibility of the technology with laser micro-dissected (LMD) cells and use the LMD to explore the minimal and optimal numbers of cells required to generate informative profiles. To do this we used the ForenSeq™ DNA Signature Prep Kit and a MiSeq FGx™ Sequencer. In this paper we describe the modifications made to our one step DNA extraction protocol to optimize sequencing performance, and address technical issues that require consideration when sequencing DNA from small numbers of cells, below the optimum amounts recommended. We demonstrate that DNA sequence profiles can be obtained from very small numbers of laser micro-dissected epithelial and sperm cells, demonstrating the utility of this approach.

10. A MICROHAPLOTYPES PANEL FOR FORENSIC GENETICS USING MASSIVE PARALLEL SEQUENCING (MPS)

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Microhaplotypes (microhaps) are defined as loci of two or more SNP within the span of a single sequencing run with three or more common allelic combination (haplotypes) of the SNPs [1-2].
Microhaps appear to be useful in forensics for individual identification, ancestry inference, estimating relationships, and deconvoluting mixtures. The most important issue is identifying and characterizing a set of microhaps with the optimum characteristics for specific purposes and developing a suitable genotyping technology.
The MPS technologies are now making microhaplotypes a new type of forensic marker: a single sequence read can cover the expanse of the microhaplotypes and these loci become phase-known (i.e. the allelic combination of multiple SNPs on each chromosome of an individual can be determined).
In the present study we selected a panel of 90 microhaps, from The ALlele FREquency Database (https://alfred.med.yale.edu/), that matched the following criteria: 1- comprised of three, four or five SNPs; 2- comprised of 2-SNPs but with Global Average Effective Number of Alleles (Ae) ranking <= 60; 3- comprised of 2-SNPs but with the Informativeness statistic for ancestry inference (I_n) ranking <= 60.
We amplified the 90 microhaps in a European population sample and performed MPS on the Ion Torrent Personal Genome Machine (PGM).
Our objectives were: to validate a robust sequencing method to separate the allelic phase; to determine, for each microhaps, the effective number and type of alleles, the allele frequencies in the population analyzed; to identify final panels, with appropriate microhaps loci, suitable for the different forensic applications.

1. Kidd KK, Pakstis AJ, Speed WC, Lagacé R, Chang J, Wootton S, Haigh E, Kidd JR: Current

sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. Forensic Sci Int Genet. 2014;12:215–24
2. Kidd KK, Pakstis AJ, Speed WC , Lagace R, Chang J, Wootton S , Ihuegbu N: Microhaplotype loci are a powerful new type of forensic marker. Forensic Sci Int Genet.Suppl. Ser. 4 2013; e123-e124

11. MASSIVELY PARALLEL SEQUENCING-ENABLED MIXTURE ANALYSIS OF MITOCHONDRIAL DNA SAMPLES

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Mitochondrial DNA (mtDNA) has several characteristics that are useful for forensic investigation. Historically, mtDNA has been analyzed with Sanger sequencing methodologies. The time-consuming, labor intensive, and costly nature of this capillary electrophoresis-based methodology has made it difficult to analyze the entire mitochondrial genome. Instead, focus has been on the polymorphic control region. Massively parallel sequencing (MPS) technologies now make it feasible for forensic crime labs to sequence the entire mitochondrial genome, which can provide an increase in discrimination power. Additionally, the quantitative nature of MPS technologies offers opportunities to resolve mixed mitochondrial DNA samples encountered in casework.
A workflow consisting of the Precision ID Whole Mitochondrial Genome Panel, Ion Chef, and Ion S5 sequencer (Thermo Fisher Scientific) was used to evaluate the ability to perform mixture deconvolution. Mixtures in a 1:1, 1:5, 1:10, and 1:20 ratio were prepared and sequenced. Performance metrics, including coverage, strand balance, and noise, were used to evaluate the quality of the sequencing results generated. Single-source reference samples included in the mixed samples study were sequenced on an orthogonal platform to evaluate concordance. The major contributor in each mixed sample was successfully identified illustrating an improvement in the ability to analyze mixed samples. Finally, the bioinformatic pipeline developed and used to analyze the mixed samples in this study will be described in an effort to aid other laboratories interested in the implementation of MPS technologies.

12. GENETIC AND GENOMIC APPROACHES TO ESTIMATING ANCESTRY

Keynote Speech

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Ancestry estimation from human DNA sequence variants has applications over a range of fields, including medical genetics, molecular anthropology, genetic genealogy and forensics. Testing has evolved from the use of sets of ancestry-informative markers (AIMs), via genome-wide short-tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) to whole-genome sequences. Markers showing sex-biased inheritance (mitochondrial DNA and the X and Y chromosomes) can reflect past sex-biased processes. Inferences from ancestry testing depend on the range and quality of comparative datasets, and also on how these are subclassified into groups. Classifications vary between fields,

including labels based on language, religion, nationality, geography, and skin-colour. Another key issue is the distinction between population-based and individual-based ancestry testing. The first can rely on the principles of population genetics, and more recent demographic modelling approaches. Inferring individual ancestry is more contentious, and yet this is what genetic genealogists and forensic scientists would like to do. Forensic autosomal STRs are poorly suited to ancestry inference because of their small numbers and high mutation rates. New next-generation sequencing multiplexes promise more power because the number of markers is larger, and because some contain biogeographic ancestry SNPs. The Illumina Forenseq system includes 22 'phenotypic' SNPs, all of which, in fact, are pigmentation SNPs. The fact that some SNPs have shifted between the 'phenotypic' and 'biogeographic' categories as the kit has evolved points to the conflation between ancestry and pigmentation, and also to the ethically concerning issue that DNA-based ancestry testing can act to reinforce the biological definition of race.

13. REVISITING THE MALE GENETIC LANDSCAPE OF CHINA

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Numerous studies have investigated China's genetic diversity. Early studies reported a genetic distinction between Northern and Southern Han Chinese, while others showed a picture of more subtle differences. Here, we investigated the distribution of Y chromosome variation across 28 administrative regions as well as 19 recognized Chinese nationalities in continental China to assess the impact of recent demographic processes. To this end, we analyzed 37,994 Y chromosomal 17-marker haplotype profiles from the YRHD database with respect to forensic diversity measures and genetic distance between groups defined by administrative boundaries and ethnic origin, representing the largest genetic study on China to date. We observed high diversity throughout across all investigated Chinese provinces and ethnicities. Kazakhs and Tibetans showed the strongest significant genetic differentiation from the Han and other groups. However, differences between provinces were, except for those located on the Tibetan plateau, less pronounced. This discrepancy is explicable by the sizeable presence of Han speakers, who showed high genetic homogeneity all across China, in nearly all studied provinces. We also observed a subtle genetic North-South gradient in the Han, confirming previous reports of a clinal distribution of Y chromosome variation and being in notable concordance with the previously observed spatial distribution of autosomal variation. Our findings shed light on the demographic changes in China accrued by a fast-growing and increasingly mobile population.

14. GENOGEOGRAPHER - A TOOL FOR GENOGEOGRAPHIC INFERENCE

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Ancestry-informative markers (AIMs) are genetic markers that give information about the genoogeographic ancestry of individuals. They are used for predicting the genogeographic origin of the investigated individual in crime and identification cases. In the exploring the genogeographic origin of an AIMs profile, the likelihoods of the AIMs profile in various populations may be calculated. However, there might not be an apt population in the database of reference populations. The fact that the likelihood ratios (LR) of one population compared to other populations are large does not imply that any of the populations are relevant. This is because that even though the populations might be exclusive, they are not exhaustive in the sense that they cover all possible human populations.
To handle this phenomena, we derive a likelihood ratio test (LRT), by which we can judge whether there is at least one population in our reference database that is "sufficiently close" to a profile's "true" genoogeographic population. The LRT is a measure of absolute concordance between a profile and a population, rather than a relative measure of the profile's likelihood in two populations (the LR).
The LRT is similar to a Fisher's exact test. The varying sample sizes of the reference populations in the database is explicitly included in the calculations, and does makes fewer assumptions than for LR calculations. The methodology has been implemented in an free open source interactive platform, GenoGeographer, that enables the forensic geneticist to make explorative analyses, produce various graphical outputs together with evidential weight computations.

15. DISCERNING THE "IDENTICAL": EXTENDED MITOGENOME DIVERSITY BEHIND THE MOST COMMON WEST EURASIAN MTDNA CONTROL REGION HAPLOTYPE

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Mitochondrial (mt)DNA is a vital tool in forensic genetics when nuclear markers do not provide results or maternal relatedness is investigated. The ~1.1 kbp non-coding mtDNA control region (CR) displays highly condensed variation and is therefore routinely typed. In this restricted range, matching haplotypes do not necessarily imply that the entire mitogenomes are identical or even belong to the same phylogenetic lineage. This is especially true for the most frequent West Eurasian mtDNA CR haplotype that occurs at a frequency of 3-4% in many European populations and is observed in numerous clades within haplogroup H ("Helena") and some HV relatives. In a seminal study, we investigated the power of massively parallel complete mitogenome 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 clades of haplogroup H and raised the power

of discrimination from 0 to >99% [1]. Here we present novel results from the ongoing investigation of an expanded pan-Italian sample of almost 300 individuals carrying the most common CR haplotype. Even with the currently ~100 samples examined so far, no saturation is reached. This study demonstrates the benefit of complete mitogenome sequencing for forensic applications to enforce maximum discrimination, highest phylogenetic resolution and more comprehensive heteroplasmy detection.

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16. THE EFFECT OF A SINGULAR POPULATION STRUCTURE ON FORENSIC ANALYSIS

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In Finland, the efficient evaluation of new DNA-markers for forensic purposes has been complicated by issues of low diversity and geographic subdivision, especially in the case of Y-chromosomal profiling. Because of the singular nature of these observed diversity patterns, it is important to further assess their geographical distribution for a more thorough understanding of their origins and history.
In order to refine the ancestry of groups within the population, the geographical patterns of mitochondrial and Y-chromosomal haplogroups of Neolithic and Mesolithic ancestry were assessed in Finnish populations. The distribution of these uniparental markers revealed a northeastern bias for hunter-gatherer haplogroups, while haplogroups associated with the farming lifestyle clustered in the southwest. In addition, a correlation could be observed between more ancient mitochondrial haplogroup age and eastern concentration. These results coupled with prior archeological evidence suggest the genetic northeast/southwest division observed in contemporary Finland represents an ancient vestigial border between Mesolithic and Neolithic populations undetectable in most other regions of Europe.
The characterization of Y-chromosomal and mitochondrial haplogroup patterns contributes to forensic science by clarifying geographical origins and further dissecting the lineages found in a homogenous population. Data gathered on Finnish population diversity can help to understand the processes affecting other small and isolated populations, thus increasing the scope to a global scale.

17. DEVELOPMENT OF ETHNIC CLASSIFICATION MODELS FOR ASIAN GROUP: A DATA MINING APPROACH

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In the genetic approach of law, genetic testing has been carried out with an emphasis on individual identification, and further studies are being conducted in an effort to obtain meaningful investigation information. Identifying ethnic groups is one of such effort. These studies have been actively carried out mainly in Europe, where there is a large movement between ethnic groups. As the socio-economic level of the Asian region has increased, and the exchange has become active, the need to distinguish the nations in Asia has also increased. In order to do this, it is necessary not only to observe differences among ethnic groups, but also to verify various measures that can be interpreted statistically appropriately. In this study, we developed three-class classification models to categorize Asian ethnic groups using data mining techniques based on Y-chromosomal short tandem repeat(Y-STR) data. In order to design a practical classification model, we considered five different data mining classification models: Decision tree, Random forest, Neural network, Gradient boosting and Ensemble model. The results showed that the classification accuracy was about 80% for each Asian group(Northeast, Southeast and Southwest Asia). It was found that the data mining techniques could play a key role in Ethnic classification problem. Attempts to these data mining approaches can be also applied for autosomes or mitochondria to build classification model. Furthermore, it has been found that the use of various statistical analyzes in the field of genetics of law can be used as a useful tool in investigation information.

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18. AN ANCESTRY-INFORMATIVE ASSAY FOR THE ASIA PACIFIC REGION

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Forensic DNA Intelligence has rapidly emerged as an important investigative tool. The ability to provide

information relating to the biogeographical ancestry (BGA) of the donor of an evidential sample would enable a more efficient use of valuable police and forensic resources and assist in decision making in the primary stages of an investigation.
Prior to the application of massively parallel sequencing (MPS) in forensic DNA analysis, differentiation of populations within the Asia Pacific region was achieved using a combination of, generally SNaPshot-based, ancestry informative (AIM) assays in multiple analyses [1-3]. As MPS enables the analysis of multiple loci simultaneously, MPS-based assays reduce consumption of limited evidential material and provide greater accuracy through the generation of extensive sequence data.
An AIM panel consisting of 160 markers was constructed with a specific focus on the differentiation of populations from South Asia, East Asia, South East Asia and Oceania in addition to differentiating African, European and indigenous American populations. The panel combines established binary SNPs with two highly informative SNP-based marker sets suited to MPS genotyping; 32 multiallelic SNPs (tri- and tetra-allelic loci) and 21 microhaplotypes, mainly Kiddlab compiled loci [4].
An Asia Pacific trial of this panel was conducted involving laboratories in this region and the Middle East. Participating laboratories contributed population samples of known ancestry. In addition to ancestry predictive performance, this study reports the component marker characteristics, panel optimisation, development of reference population data sets and the adaptation of ancestry-inference analysis tools for the analysis of multiallelic loci.

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19. PREDICTION OF ASIAN ETHNIC SUBGROUPS USING HID-ION AMPLISEQTM ANCESTRY PANEL

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Prediction of ethnicity in Asia can be approached in a variety of ways, but the method using Ancestry Informative Markers (AIMs) provides additional information. The HID-Ion AmpliSeq™ Ancestry Panel is a forensic multiplex platform consisted of 165 autosomal markers designed to provide biogeographic ancestry information.
In present study, we have investigated 750 unrelated Asians, from southern China (n=99), Beijing (n=100), Japan (n=101), Korean (n=100), Vietnam (n=100), Nepal (n=100), India (n=51), and Pakistan (n=99). The Torrent Server and the HID SNP genotyper plugin provide the calculated ethnicity probability and likelihood ratio. However, a variety of statistical approaches are needed when considering that Asians are closely related geographically and historically and there is not enough data available. For this reason, several statistical techniques have been tried and compared with the results provided by the plugin. Also, we applied various statistical algorithms for ethnic that classify Northeast Asian, Southeast Asian (Vietnamese) and Southwest Asian using SNP data from panel.
This research is meaningful in terms of the sub-classification of Asian people and applicability without major changes whenever new population is added. Furthermore, if further research is continued from

the viewpoint of usability when considered together with Lineage markers, it is expected that it will provide more comprehensive information.

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20. INCREASING THE NUMBER OF RAPIDLY MUTATING (RM) Y-STRS SIGNIFICANTLY IMPROVES MALE RELATIVE DIFFERENTIATION

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Y-chromosomal Short Tandem Repeats (Y-STRs) are valuable markers in forensic casework, particularly suitable for solving sexual assault cases, where the excess of female DNA often obscures male perpetrator identification when using standard autosomal DNA markers. Commonly used Y-STR sets, including those available via commercial kits, provide high, but not maximal, differentiation of unrelated men, and typically cannot differentiate between related men. Aiming to overcome this limitation of forensic Y-STR analysis, we previously introduced and further characterized a set of 13 Rapidly Mutating (RM) Y-STRs. In a recent multicenter study, these 13 RM Y-STRs achieved male lineage differentiation of >99% and male relative differentiation of >29% on average, which is superior to any other Y-STR set currently available. Aiming to find new, additional RM Y-STRs for further increasing male relative differentiation, particularly regarding closely related men, we performed in-silico search of the human Y-chromosome sequence for suitable candidate markers. We developed multiplex systems for the most promising candidate markers, and genotyped them in various pedigree and family samples to estimate relative differentiation rates. Our newly identified set of RM Y-STRs performed similarly well as our previously introduced RM Y-STR set. Considering RM Y-STRs from both sets together, we achieved a significant improvement of male relative differentiation, including close relatives, compared to previous findings. We therefore present a big step towards male individualization from DNA by keeping with the forensic advantage of Y-STR analysis to solve cases involving mixed male-female traces.

21. GENOMICS APPROACHES FOR CHARACTERIZING BIOLOGICAL DIVERSITY: FROM IDENTIFYING SPECIES TO MONITORING WHOLE ECOSYSTEMS

Keynote Speech

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Rapid advancements in genome technologies and computational tools have revolutionized bio-medical sciences. The sheer volume of sequence information generated by a single DNA sequencing platform

has significantly increased in less than a decade. Most of this sequence information encompass a small number of organisms with human and their disease models such as mouse being among the most sequenced species of eukaryotes. Aside from assembling a complete (whole) genome sequence from an organism, most investigations focus on specific segments of the genomes. For example, research has shown the usefulness of comparative genomics for identification of units of biological diversity. Similar to DNA fingerprinting approaches that are common in forensic analysis, comparative analysis of a small number of genes can provide a basis for taxonomic identification of all species from microbes to mammals. Over the last decade this "DNA barcoding" approach has gained much momentum and its global sequence database includes over 5M records from 0.5M species. Consequently, the use of sequence information in identification of species has aided challenging problems such as environmental assessment, authentication of food and natural health products, forensic investigations as well as detection of pests, pathogens and species at risk. Additionally, bulk DNA extracted from environmental samples such as soil or seawater have been shown to provide comprehensive biodiversity information at ecosystem level. The widespread availability of genomics technologies and their automation-friendly nature further aid their rapid adoption by various stakeholders from academic researchers to regulatory agencies to any person interested. The integration of genomics in biodiversity analysis will transform our ability to detect and understand biological diversity of any group of organisms at any setting. This will set the stage for a bio-literate society.

22. 'FORCYT' DNA DATABASE OF WILDLIFE SPECIES

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The illegal trade in highly endangered species continues unabated. High profile species such as rhinoceros, elephant and tigers are on the brink of extinction in many parts of the world. Robust and reliable forensic science based on DNA sequence comparisons are required to enforce national legislation.

We report on the establishment of ForCyt, a DNA database to aid with prosecutions of illegally traded species. We will present in detail the need for ForCyt and how it will assist in wildlife forensic science.

Current practice compares a questioned DNA sequence to a database. The largest database, GenBank, has a wealth of DNA data but is not regulated and rarely are the DNA sequences from voucher specimens.

ForCyt will be established based on regulated and checked DNA data with supporting information. This information will include: confirmation of correct species identification; up-loaded pictures are provided; any additional means of identification (stud book/microchip); date of collection; the geographic origin of the sample; preservation of sample; integrity of any packaging.

Information of the laboratory practice will be required including: persons performing the analyses; method of any DNA extraction performed; designation of primers used; site of DNA sequencing (in-house or out sourced); and whether the laboratory followed GLP or is accredited to a particular standard. All sequence data will be subject to checking for ambiguities and anomalies.

The outcome will be a repository of validated DNA sequence data to which others may make comparisons. Robust conclusions as to the species origin of samples can be made.

23. NON-HUMAN FORENSIC IDENTIFICATION OF COMPLEX MIXTURE SAMPLES USING DNA METABARCODING

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DNA identification is a powerful tool and has been widely used in forensic genetic investigations. Recently, the scope of DNA identification has been expanded with the introduction of massive-parallel sequencing (MPS) technology. DNA metabarcoding is a high-throughput approach of quickly and cheaply generating the barcodes of large number of species to be sequenced together in a single reaction. Several universal PCR primers can even be applied to simultaneously detect bacteria, animal, plant, and so on. In order to improve genetic identification of non-human forensic samples, we have developed a forensic database system to perform complex analyses of large datasets and translate the results of these analyses. The ForensicBOL (Forensic Barcode of Life) is an integral database system which analyzes and manages both DNA barcoding and metabarcoding data. Here we report two non-human forensic cases using DNA metabarcoding with universal DNA barcode markers. In the first case of the illegal drugs in a capsule form, we identified a variety of raw materials such as plants and animals (including humans). In the second case, known as the "Fake Back-Su-O" case, we detected a prohibited plant, *Cynanchum auriculatum*, and accurately calculated mixture ratio from the dietary supplements in the form of powders, dried products, and teas. DNA metabarcoding is a promising identification approach to detect prohibited and hazardous species in complex forensic samples. This approach for species identification would widely expand our ability of genetic screening in various non-human forensic cases.

24. CANINE STR MARKER GET MORE – DOG BREED AFFILIATION WITH A FORENSICALLY VALIDATED STR PANEL

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In many aspects of human social life, dogs (Canis familiaris) play a major role and, therefore, they occupy a significant niche as a source of forensic samples. Dogs can cause accidents or act as perpetrators of attacks, but the transfer of canine DNA evidence that allows victims, suspects, and crime scenes to be linked, can be regarded as even more important. Typically – and in line with human DNA identification approaches – the identification of the "dog of interest" is mainly based on STRs. The Canine DNA Profiling (CaDNAP) Group, a long-standing collaboration between institutes in Austria, Germany and Switzerland, established a panel of 13 forensically validated STR-markers. This panel has repeatedly demonstrated its capability to identify individual dogs. However, in cases where DNA profiles do not match a specific dog, other sources of evidence such as breed membership may gain importance. Due to the genetic structure of dog breeds it is well established that such assignments based on STRs are generally feasible, but a proof of concept in the forensic context is still missing.
We tested the CaDNAP-STR-panel for breed assignments using samples from breeds which are

forensically relevant and common to Austria, Germany and Switzerland. Therefore, a survey of purebred and crossbred dog frequencies was performed in advance, as the analyzed samples should adequately mirror a realistic dog population. Statistical methods applied include Bayesian clustering, population assignments, and discriminant analysis of principal components (DAPC) provided by software programs such as "STRUCTURE" and the "adegenet" R package.

25. IDENTIFICATION OF HOAX AGENTS, *BACILLUS* AND *YERSINIA* STRAINS BY PROTEIN PROFILING USING MICROFLUIDIC CAPILLARY ELECTROPHORESIS WITH PEAK DETECTION ALGORITHMS

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Bacillus anthracis and *Yersinia pestis* are biological agents that pose an increasing concern to national security, if deliberately disseminated. Hoax agents including suspicious white powders and environmental bacterial species can also cause disruption. In either scenario it is of high importance to rapidly and accurately identify any suspicious powder as hazardous or hoax. This can be achieved using protein profiling by microfludic capillary electrophoresis as a field-based automated screening method. Two commonly encountered hoax agents (Dipel containing *B. thuringiensis* and plain flour), three *Bacillus* species (*B. anthracis* Sterne strain, *B. thuringiensis* Kurstaki strain and *B. cereus*), two *Yersinia* species (*Y. enterocolitica B1A S09* and *Y. pseudotuberculosis*) and *E. coli* were profiled using microfluidic capillary electrophoresis on the Experion™ System (Bio-Rad).
Peak detection algorithms allowed for the recognition and identification of protein peaks in raw electropherograms. Boolean logic gates were then employed to model and predict the electrophoretic protein pattern of species based on the presence or absence of indicative protein peaks in specified time windows. Parameters assessed included variation within and between Experion™ Pro260 chips and ability to identify and discriminate between species over time intervals, between operators and between field and laboratory analyses. The results indicate that this technique is rapid (less than 40 minutes), accurate and reproducible for automated front-line identification and differentiation of hoax, biological and environmental agents.

26. MICROBIOME COMMUNITIES ANALYSES TO PREDICT BODY SITES: ANALYSES ON PUBLISHED DATA AND ON EXPOSED SAMPLES IN THE LABORATORY

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Human microbiome sequencing studies have shown that microbial communities across body habitats are distinct, and therefore potentially useful for the forensic prediction of body sites from fluids or stains. However, studies examining body site classification methods have focused on datasets from single studies, and have generally examined single sources only, while forensic samples are frequently mixtures. We examined the performance of random forest classifiers on a large and heterogeneous dataset comprising publically available 16S rRNA gene sequences from 15,082 saliva, skin, nostril, vagina and feces samples from 57 studies. Additionally, we also produced *in silico* mixtures of sequences for all pairs of body sites, testing the prediction accuracy of the classifiers for different ratios. Our results indicate high prediction performances for the single source sites. We were also able to reliably identify both components in mixtures with fractions as low as 2% for the minor body site. These results are promising, and highlight the potential of microbial 16S rRNA gene sequencing for forensic applications. However, forensic samples are frequently exposed to environmental conditions and therefore subject to degradation. Therefore, we have also initiated a study investigating the differences in the microbial compositions of a total of 36 skin, saliva, menstrual blood, peripheral blood, vaginal fluid and semen samples that have been exposed in the laboratory for 4 weeks. We highlight the main differences across freshly extracted and exposed samples, and the predictive accuracy of our classification methods.

27. MICROHAPLOTYPE: ABILITY OF PERSANAL IDENFICATION AND BEING ANCESTRY INFORMTIVE MARKER

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Microhaplotype is a new type of genetic marker. Due to the multiallelic characteristic, microhaplotype could have much better personal identification ability compared to the bi-allelic SNPs. Microhaplotype could also have alleles associated with specific populations. With this research, phased data from 1000 genomes project were used to evaluate the ability as personal identification and ancestry informative marker. Totally 32 previously selected microhaplotype loci and 568 unrelated individuals from six region around the globe were enrolled. Including Africans, Europeans, East Asians, Americans. Specifically from African Caribbeans in Barbados (ACB), Americans of African Ancestry in SW USA (ASW), Northern and Western European Ancestry (CEU), Han Chinese in Beijing, China (CHB), Southern Han Chinese (CHS) and Japanese in Tokyo, Japan (JPT). The range of SNPs number per locus among the 32 loci has a range from 3 to 10. The allele number range of the 32 microhaplotpype is 3 to 26. The median heterozygosity for these 32 loci is 0.6717 for the 6 populations studied and ranges from 0.5461 to 0.8693. To assess the power as AIM, we applied standard population analysis approaches of the Nei's genetic distance and STRUCTURE analysis. The genetic distance generated by 32 loci demonstrated that ACB and ASW as well as CHB and CHS were conglomerated together. 6 loci were picked out to run the STRUCTURE analysis for an optimum K:4. Visual inspection of cluster plots suggests a close match between the co-ancestry patterns seen in ACB and ASW as well as in CHB, CHS and JPT.

28. MICROHAPLOTYPE FOR ANCESTRY PREDICTION

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Microhaplotypes (MHs) are loci of two or more SNPs within a short distance from each other (<300 nucleotides) with three or more allelic combinations1. Massively parallel sequencing (MPS) methods, by allowing clonal sequencing of individual strands, can distinguish the parental haplotypes at a locus. MH alleles within a locus all have the same size, do not generate stutter fragments, and have lower mutation rate than STRs. The goal of this project was to evaluate if MHs could provide biogeographic ancestry prediction.
One hundred (100) European-Americans (EAs), 50 African-Americans (AAs), and 92 Southwest Hispanics (SWHs), were analyzed with a 33 MHs panel on Ion S5™ platform. PHASE inferred allele frequencies from 58 populations2 were used to calculate the random match probability (RMP) of each profile in each population. The RMP averaged higher in the populations individuals self-identified as. For example, AAs had the highest RMP in African and African-American populations.
Likelihood ratios (LR) were calculated by dividing the highest RMP obtained from the three US populations by the second highest in that same set. The number obtained represented how much more likely it is to observe that profile if the individual is of the population at the numerator vs the one at the denominator. All individuals were predicted of the correct population except for 38% of SWHs, which were not surprisingly predicted as EAs given it is an admixed population. The 33 MHs were not selected for informativeness, more specifically selected markers will likely improve the prediction's accuracy.

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2. Evaluating 130 microhaplotypes across a global set of 83 population. Kidd, Kenneth K. et al. Forensic Science International: Genetics , Volume 29 , 29 - 37

29. THE APPLICATIONS OF MALAYSIAN POPULATIONS PLOTTED VIA NEXT GENERATION SEQUENCING

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Next Generation Sequencing (NGS) is a brand new approach in forensic DNA analysis where information contained in DNA evidence is materialized such STRs, Phenotypic SNPs such as hair colour, eye colour and skin colour; ancestry and also identity SNPS which is suitable for DVI cases are utilized to identify unknown criminals. This technique generates much more data compared to the current 16 loci adapted in our laboratory. Malaysian population originated from three main ethnicities which is the Malaysian Malay, Malaysian Chinese and Malaysian Indian were analyzed using the MiSeq FGx Next Generation Sequencing.
In May 2015, Malaysia's Wang Kelian villagers were shocked by discovery of 139 mass graves found in jungles of Perlis near the Thailand border, believed they were the victims of human trafficking

syndicates. The remains removed from the jungle were a highly decomposed body that had been left out there for over couple of weeks. A total of 122 mass grave samples which consist of 119 bone samples and 3 teeth samples were received. The results showed that mass grave samples were not clustered in either Malaysian Malay or Malaysian Chinese population but originated from Indian population. However, after analyzing the reference sample of Rohingyas individuals the results indicated that these mass grave samples are likely Rohingyas rather than Malaysian.

30. COMPLETE MITOCHONDRIAL GENOMES OF THE HUNTER-GATHERER POPULATIONS IN THAILAND

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Hunter-gatherers are generally assumed to be maintaining the genetic lineages of anatomically and behaviorally modern humans who dispersed out of Africa at least 50,000 years ago. Part of the rationale of the assumption is the presence of basal mitochondrial (mt) DNA lineages in many hunter-gatherer populations outside Thailand, for example in the Andaman Islands, Malaysia, and the Philippines. There are two hunter-gatherer groups, i.e., the Mlabri and the Sakai, living in northern and southern Thailand, respectively. We sequenced full mitochondrial genomes of 18 and 11 unrelated subjects of Mlabri and Mani. Only 2 haplotypes differing by a single polymorphic site are found in the Mlabri, and all sequences belonged to haplogroup B5a1b1. We found 5 haplotypes in the Mani belonging to 3 basal haplogroups, that is, M17a, M21a and R21. The reduced genetic diversity of these two populations results in increased genetic differentiation when compared to other populations from Thailand. The coalescent age of the two Mlabri haplotypes is 735 years, suggesting that the Mlabri have reverted to a hunter-gather lifestyle, in agreement with a previous study (1). However, the Mani share maternal lineages with other hunter-gatherer populations from outside Thailand, suggesting that they are indigenous hunter-gatherers.

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31. DNA TRANSFER AND CELL TYPE INFERENCE TO ASSIST ACTIVITY LEVEL REPORTING

Keynote Speech

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Courts are often concerned with interpretation of evidence beyond the proposed identity of an individual, and questions such as ‘what cell material is present and how did it get there?’ need to be addressed [1]. RNA profiling has developed into an more and more accepted methodology to infer cell types present in evidentiary material. Two types of multiplex assays exist: those typing body fluids and

another targeting organ tissues. These assays find their predominant application in sexual assault and violent cases respectively, which will be illustrated by casework examples. Alternatively, DNA methylation may provide useful information to assist cell type inference, markedly when semen is involved. To provide context to observations, subjects such as background, transfer, persistence and recovery require data [2] for which an illustration will be given. Such information will assist interpretation for which commonly hypotheses-based approaches are used.

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32. ASSOCIATION OF A BODY FLUID WITH A DNA PROFILE BY TARGETED RNA/DNA DEEP SEQUENCING

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The identification of body fluids/tissues is an important part of forensic casework and can contribute valuable information about the circumstances of a crime.
Our goal for this study was, in addition to body fluid/tissue identification, to associate tissue specific mRNA transcripts to the donor of a stain. Within the tissue specific sequences we looked for sequence variants (SNPs) that discriminate Caucasian individuals the most. We successfully tested a targeted NGS assay for the Illumina MiSeq platform in 188 European individuals for the detection of the 35 selected SNPs on gDNA level. To assess the usefulness of these 35 markers we estimated how powerful the loci are to discriminate between individuals. The match probabilities for the SNPs within one body fluid and for sets of markers from different body fluids confirmed the discriminatory power of these markers. A separate targeted cSNP panel on RNA level showed good specificity for blood, semen and menstrual blood. For saliva, vaginal secretion and skin, the marker design needs to be optimized with special attention to cross reactivity with DNA contaminants. As a proof-of-principle we could demonstrate that with our targeted DNA and RNA NGS assays we are able to assign a body fluid to a specific individual. This is especially interesting for mixed body fluid samples, where we can find out who contributed which body fluid.

33. DIRECT PCR IMPROVES STR PROFILES FROM SUBSTRATES OF IMPROVISED EXPLOSIVE DEVICE

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STR profiling from both pre-blast and post-blast IED evidence are generally unsuccessful due to the low-level of DNA present and high level of degradation. Previous studies have improved the number of alleles recovered by focusing on both the DNA collection process and the downstream processes of

DNA extraction and amplification; however, direct PCR has never been used with touch DNA on IED evidence. In this study, we optimized a direct PCR protocol for touch DNA profiling from various IED substrates. Two swab types with various moistening agents and one type of tapelifting were compared as the collection methods. Different amounts of cuttings from swab heads and tapes were evaluated. Two direct PCR protocols – the direct protocol and the pre-PCR protocol – were performed. As expected, different substrates (i.e. clothes, PVC pipe, circuit board, and electrical tape) required different collection methods and cuttings for optimal DNA recovery and amplification. The direct protocol recovered significantly more alleles than the pre-PCR protocol ($p<0.05$). The optimal direct PCR methods were compared with our standard operating protocol (conventional DNA extraction and STR typing) using mock IED evidence. This improvement could be due to the omission of the inefficient DNA extraction step, which in turn reduces the cost of STR profiling. The result showed that more alleles were recovered using the optimal direct PCR methods ($p<0.05$). The findings of this study suggest that direct STR typing could be used IED evidence, provided that an appropriate, optimal DNA collection method is used.

34. THE DEVELOPMENT OF A FORENSIC CLOCK TO DETERMINE TIME OF DEATH

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The time and date of a person's death are two major questions police detectives and investigative judges ask a forensic pathologist as it provides a time frame in which the investigators can base their further investigations on. The current time of death (ToD) estimation techniques produce large error ranges since environmental and endogenous factors have to be taken into account. Too large prediction intervals for ToD estimation are of little use to the investigators though at present no tools are available to limit the ranges offered.
Circadian biomarkers (cortisol, melatonin and 3 mRNAs) have already proven their potential use as forensic molecular clock markers to estimate blood-deposition time [1], and intriguingly circadian biomarkers identified from the brain transcriptome were previously shown to be able to estimate ToD with a ± 1.9h error range [2]. The application of circadian biomarkers to estimate ToD would therefore mean a significant improvement in accuracy compared to current techniques, especially after the first 24h after death, as error ranges increase exponentially with increasing postmortem interval.
In order to develop a forensic molecular clock for postmortem blood samples we designed a study to identify circadian markers in three different classes of molecules using postmortem blood samples obtained from deceased individuals with a known ToD (±30 min): circadian transcripts with RNA-seq, circadian metabolites with LC-MS/MS and melatonin and cortisol concentrations using ELISA kits.
During this presentation we will discuss our initial results and demonstrate the applicability of circadian biomarkers for the determination of time of death.

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2. Li JZ, Bunney BG, Meng F, Hagenauer MH, Walsh DM, Vawter MP, et al. Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. Proc Natl Acad Sci U S A. 2013;110(24):9950-5

35. LATENT DNA DETECTION USING FLUORESCENT DNA BINDING DYES

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We report on the use of DNA binding dyes that can target the collection of DNA present on samples. Dyes are available that bind to DNA at high specificity for laboratory-based applications but rarely applied to *in situ* detection.
Common nucleic acid-binding dyes were selected due to their increase in fluorescence when in the presence of DNA (SYBR® Green I, Diamond™ Nucleic Acid Dye, GelGreen™, GelRed™, EvaGreen™ and Redsafe™). The fluorescence from dye/DNA complex was detected using a high intensity light source, the Polilight® (PL500), and emission observed through band pass interference filters with a 40nm band width and central wavelength of 530 nm or 550 nm depending on the dye emission. Some biological samples such as hair and skin were visualised under a fluorescent microscope (Nikon Optiphot) using a B2A filter cube.
Detection of DNA was observed within different biological samples such as saliva, skin, blood and hair which make it possible to select samples that are more likely to produce STR profiles after direct amplification. The use of these dyes as a screening methodology for hairs was conducted where the fluorescent signal was correlated with the quality of STR profile obtained.
The outcome of this work is an innovative means to detect DNA *in situ* within biological samples and on surfaces that make the screening of samples more efficient and successful. The investigation so far has concluded that EvaGreen™ and Diamond™ dye are the optimum dyes for this novel application based on their properties of binding and limited interactions with downstream forensic applications such as DNA extraction, amplification and STR typing.

36. ASSESSMENT OF THE PREVALENCE, TRANSFER, PERSISTENCE AND RECOVERY OF DNA TRACES FROM WORN UPPER GARMENTS: RESULTS FROM AN INTER-LABORATORY STUDY

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Clothing items are commonly recovered from crime-scenes and submitted for DNA analysis and subsequent interpretation. Depending on the case circumstances, sampling of the garment may target the wearers DNA or the transferred DNA of one or more offenders. In addition to collecting targeted DNA, traces of background DNA from sources unrelated to the crime event, may also be inadvertently collected. This has the potential to raise questions regarding the relevance of traces. To gain an

understanding of the prevalence, transfer and persistence of DNA on clothing after normal washing and wearing scenarios, samples were collected from several areas of upper garments worn by individuals on working or non-working days, and processed from DNA extraction through to profiling. Activities relating to the garment prior to and during wearing were recorded by the wearer through questionnaires. Reference profiles were obtained from the wearer and their close associates identified within these questionnaires. This was repeated for sixteen individuals across four countries, and the impacts of differences among collaborating laboratories in sample collection through to profiling were considered during the interpretation and analysis of the 400+ profiles generated. Variations in the number of contributors, the composition of the profiles, and inclusion/exclusion of the wearer and their close associates were observed between the collaborating laboratories, participants, garments worn on different occasions, garment types and garment areas sampled. Impacts of activities undertaken during wearing were also observed.

37. DNA TRANSFER STUDY BASED ON PARALLEL SYSTEM METHODS

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With the ability to detect small amounts of DNA, just a few cells, the occurrence of DNA transfer challenges the probative value of this type of evidence [1]. Several studies have demonstrated factors affecting the occurrence and probability of DNA transfer (for example 2), but these data sets cannot be easily generalized and used to assess transfer in specific court cases. We are proposing the use of complex system modeling to systematically re-examine DNA transfer processes and related research data, establishing relevant parameters. A crime is essentially an open complex system of interactions in an environment, thus the DNA transfer process, which presents spatial-temporal uncertainty and behavioral complexity, can be modeled as such. The ACP approach [3], a combination of artificial societies (A), computational experiments (C), and parallel execution (P), can be used to create a parallel DNA transfer system and model the different factors and interactions. This method has the potential to provide a strong scientific basis and decision support when dealing with DNA transfer issues.

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38. CPGFORID: EPIGENETIC DISCRIMINATION OF IDENTICAL TWINS UNDER THE FORENSIC SCENARIO

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Standard DNA profiling is typically non-informative for differentiating between monozygotic (MZ) twins. Whole genome sequencing was recently proposed as a solution; however, both the rarity of such autosomal SNPs and required ultra-high coverage resulting in immense costs, make this approach impractical. Epigenetic variation was identified as suitable source for MZ twin differentiating biomarkers; nevertheless, studies addressing the forensic feasibility are lacking. Here, we performed the first use of DNA methylation for MZ twin differentiation under the forensic scenario, comprising the i) discovery of twin-differentially methylated sites (tDMSs) in reference-type DNA via genome-wide analysis, ii) validation of candidate tDMSs using forensically suitable, targeted methods, and iii) analysis of validated tDMSs in trace-type DNA. Whole blood and buccal cells of 10 MZ twin pairs were analyzed together with forensic-type material including minute bloodstains and cigarette butts. Genome-wide analysis using HumanMethylation450 BeadChips (Illumina) covering 2% of all CpGs, identified hundreds of twin pair-specific tDMSs. Analyzing the top tDMSs per pair using methylation-specific quantitative (q)PCRs in reference-type DNA confirmed many, but not all, markers. Testing the validated tDMSs in trace-type DNA revealed the observed methylation differences for many, but not all, markers. Demonstrating that a proportion of tDMSs selected from reference-type samples eventually becomes non-informative in trace-type samples questions the number of markers needed for successful MZ twin differentiation and identification. Aiming to overcome restrictive marker selection via epigenome screening of both reference and trace-type samples, we applied a novel, highly sensitive enzymatic-based methylation DNA sequencing approach (MeD-seq) covering ~50% of all CpGs.

39. APPLICATION OF DIP-STRS TO SEXUAL/PHYSICAL ASSAULT INVESTIGATIONS: EIGHT CASE REPORTS

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DIP-STRs are compound markers formed by a deletion/insertion polymorphism (DIP) linked to a microsatellite (STR). They enable the deconvolution of unbalanced DNA mixtures from two individuals, up to 1,000-fold excess of the major contributor (1-2). Here, we report on the first use of these new markers in casework to discuss their advantages and limitations in forensic practice.
Trace samples suggestive of containing unbalanced DNA mixtures were selected from eight cases with the permission of the Police and General Prosecutor authorities. DIP-STR results contributed to each case, either by complementing Y-STRs results or by producing novel investigative leads. This was especially the case when considering same sex unbalanced DNA mixtures, female minor/male major unbalanced DNA mixtures or to discriminate two brothers in a sample containing high levels of female DNA. Positive results were obtained at 16,000-fold excess of major DNA using few picograms of input DNA as well as from traces collected several months after an aggression. Likelihood ratios assigned to DIP-STRs' results were modest (10), when based on two markers, and high (106) when determined on six markers. In some cases the detection of extra alleles from additional minor DNA contributors or the observation of stochastic effects with low-template DNA samples, limited the interpretation of the results. In conclusion, the DIP-STRs often provide additional value to the analysis of traces that cannot be exploited by the use of standard methods.

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DNA mixtures. Forensic Science International: Genetics 19:156-64 (2015)

40. ADVANCES IN HUMAN APPEARANCE GENETICS AND RELEVANCE FOR FORENSIC DNA PHENOTYPING

Keynote Speech

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Retrieving appearance details of an unknown trace donor from his/her DNA left behind at a crime scene can be useful information to focus police investigation towards finding unknown perpetrators of crime, who typically cannot be identified via standard DNA profiling. Likewise, establishing appearance information from DNA obtained from human remains can be useful for finding potential relatives in missing person cases where no ante-mortem samples or relatives are available. Over recent years, this notion has led to the establishment and further development of Forensic DNA Phenotyping, where phenotypes in the closest sense refer to externally visible characteristics, and in the wider sense additionally include life time age and bio-geographic ancestry also providing investigative information. Currently it is possible, based on genetic knowledge generated and used to develop and forensically validated suitable genotyping and statistical tools, to predict categorical eye, hair, and skin color from crime scene DNA with accuracies deemed suitable for practical applications. Recent activities towards understanding the genetic basis of human appearance comprehensively concern pigmentation traits on more detailed level, various hair traits including head hair structure, head hair loss particularly in men, eye brows, as well as some other externally visible traits. This keynote speech will summarize recent progress in finding genes underlying human appearance and will discuss their relevance in the context of Forensic DNA Phenotyping.

41. EPIGENETIC AGE SIGNATURES IN SALIVA: AGE PREDICTION USING METHYLATION SNAPSHOT AND MASSIVE PARALLEL SEQUENCING

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DNA methylation is one of the most promising markers of age prediction. Many DNA methylation-based age predictive models have been developed based on DNA methylation patterns from blood. However, few studies have aimed to predict age from saliva, which can be frequently found at crime scenes. In this study, we generated genome-wide DNA methylation profiles of saliva from 54 males and identified 6 CpG markers on the *SST*, *CGNA3*, *KLF14*, *TSSK6*, *TBR1*, and *SLC12A5* genes that showed a high correlation between methylation and age. With 226 bisulfite-converted saliva DNA samples, we investigated DNA methylation at 6 age-associated CpGs as wll as a cell type-specific CpG from the *PTPN7* gene using the methylation SNaPshot method. Then we constructed an age-predictive model with the age information and the methylation profiles from the 113 training samples. The model showed

a correlation between predicted and chronological age of more than 90%, and a mean absolute deviation from chronological age (MAD) of 3.13 years. Subsequently, the validation set composed of the remaining 113 samples presented a 95.2% correlation between the predicted and chronological age, and a MAD of 3.15 years. Our model based on DNA methylation profiling using multiplex methylation SNaPshot is expected to be easily integrated into the routine forensic laboratory workflow. Also, since the suggested 7 CpG sites could be applied to other platforms through appropriate modeling, we additionally analyzed our proposed CpG sites using Massive Parallel Sequencing and tested age predictability using a newly trained model.

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42. EPIGENETIC PREDICTION OF AGE IN DIFFERENT STUDY GROUPS

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Insight into the aging rate of individuals may provide a more precise prediction of progressive appearance traits. DNA methylation is considered to be a measure of the cumulative effect of the body's epigenetic maintenance system, suggesting that the aging process may be accelerated by many factors affecting epigenetic stability. Therefore, identification of these factors could be important for the accurate prediction of age, particularly in older individuals, where epigenetic prediction is more prone to error. On the other hand, some studies suggested different sensitivity of DNA methylation loci to environmental factors and showed that, e.g. ELOVL2 methylation increases with cell divisions and to a lesser degree is influenced by other factors. In the present study we address the problem of age prediction accuracy in different individuals by testing DNA methylation measured with pyrosequencing technology using the predictive capacity of five age-related markers (ELOVL2 c7, C1orf132 c1, FHL2 c2, TRIM59 c7, KLF14 c1). Analysis involved several groups including patients of Early Onset Alzheimer's Disease, Late Onset Alzheimer's Disease, patients with Graves' Disease and a group of professional athletes. Our study showed altered DNA methylation patterns and prediction capacity in the tested groups when they were compared to healthy controls in the three markers: TRIM59, KLF14 and FHL2; suggesting their sensitivity to various factors affecting methylation age of an individual. Two important DNA methylation markers: ELOVL2 and C1orf132 were found to correctly predict age in all the tested groups, confirming their power and robustness as chronological age predictors in forensics.

43. ASSOCIATION BETWEEN BMP4 GENE POLYMORPHISMS AND EYELID TRAITS IN CHINESE HAN POPULATION

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Forensic DNA phenotyping (FDP) refers to the prediction of unknown sample donors' externally visible characteristics (EVCs) using only genetic information from extracted DNA. And several phenotypic trait estimations have been applied to forensic casework. FDP for single/double eyelids could be quite useful in forensic practice, especially in East Asia. To evaluate the association between polymorphisms of BMP4 gene and eyelid traits, a case-control study including 611 Chinese Han adults was conducted and two candidate SNPs were genotyped using PCR-RFLP. Significant differences were found for rs2761882 CT and CT/TT genotypes in single and double eyelids populations ($p<0.05$); no difference was observed for rs762642 ($p> 0.05$). Single eyelid was further divided into four subgroups: normal eyelid (123 cases), eyelids with thin and soft skin (28 cases), eyelids with thick subcutaneous tissue (28 cases) and eyelids with triangle eyes (27 cases). Typical double eyelids also had four types, including moderate (140 cases), narrow (106 cases), wide (24 cases), a wide and a narrow (42 cases) types. The classification for untypical double eyelids referred to typical double eyelids: moderate (34 cases), narrow (19 cases), wide (22 cases), a wide and a narrow (18 cases) types. Stratification analysis showed significant difference of rs2761882 CT genotype frequency between a wide and a narrow untypical double eyelids and the other subgroups. Significant difference of rs762642 GG genotype frequency also has been found between a wide and a narrow typical double eyelids and the other types. Further analysis is still in process.

44. DISCRIMINATING BETWEEN UNCLE-NEPHEW AND GRANDFATHER-GRANDSON KINSHIPS BY ANALYZING CHROMOSOMAL SHARING PATTERNS

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We developed a new method for pairwise kinship analysis using the index of chromosomal sharing from high-density single nucleotide polymorphisms (SNPs) that was established in our previous study [1]. Using the previous method, we could determine accurate degrees of kinship up to the third-degree in cases where no predicted relationship existed between two individuals. However, discriminating between kinships of the same degree (e.g., uncle-nephew and grandfather-grandson) was difficult. In the present study, we examined the differences in chromosomal sharing patterns between uncle-nephew and grandfather-grandson kinships.
We computationally generated genotypes of 174,254 autosomal SNPs in 249 uncle-nephew and grandfather-grandson pairs, while considering the effect of linkage disequilibrium among each SNP. We examined the shared chromosomal segments, using our previous method [1], and counted them to investigate the differences in chromosomal sharing patterns. We then classified these chromosome

segments, using a receiver operating characteristic curve analysis. We confirmed the validity by using actual data.
The median values of the number of shared segments in uncle-nephew and grandfather-grandson kinships were 63 and 39, respectively. The area under the curve was 0.99954, indicating that the two relationships were clearly different. Moreover, the actual sample pairs showed similar results. These results suggest that shared chromosomal segments in collateral relationships are greater in number but smaller in length than those in lineal relationships with the same degree of kinship.

1. Morimoto C et al: Pairwise kinship analysis by the index of chromosome sharing using high-density single nucleotide polymorphisms. PLoS One. 2016; 11: e0160287

45. A NEW (APPROXIMATE) METHOD FOR Y-STR HAPLOTYPE PROBABILITY ASSIGNMENT

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All current statistical models for the interpretation of Y-STR mixtures are hampered by a single point of failure–namely that there is no single agreed model for the assignment of Y haplotype probabilities. There are numerous competing models: the counting method, Brenner's kappa method, the generalized Good method, the coalescent, the discrete Laplace, each with their strengths and weaknesses. In this talk we introduce a new approximate method, that can build probabilities locus by locus. We will discuss the basis for its derivation and show how it may have some grounding in traditional information theory. Finally, we will compare its performance to existing methods.

46. HOW CONVINCING IS A MATCHING Y-CHROMOSOME PROFILE?

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The introduction of forensic autosomal DNA profiles was accompanied by much controversy over how to evaluate the weight of evidence. The problems have been successfully addressed, and DNA profiling has gone on to revolutionise forensic science. Y-chromosome profiles are valuable when there is a mixture of male-source and female-source DNA, and interest centres on the identity of the male source(s) of the DNA. The problem of evaluating evidential weight is even more challenging for Y profiles than for autosomal profiles. Numerous approaches have been proposed; we show them to be unsatisfactory. Men with matching Y-profiles are related in extended patrilineal clans. The available databases will not represent many of these clans, while others may be over-represented due to sampling biases. Further, the population of forensic relevance is difficult to define, and the number of matching relatives is fixed as population size varies, which means that population-based match

probabilities are of limited value. We propose a very different and surprisingly simple solution to the problem. We provide a simulation model and software to approximate the distribution of the number of males with matching Y profiles. The distribution can be modified to condition on a count from a randomly-sampled database, if available. Our approach allows, for the first time, the value of Y profile evidence to be explained to a court in a way that is scientifically valid, quantitative and easily comprehensible by a finder of fact such as a judge or juror.

47. USING EUROFORMIX TO ANALYSE COMPLEX SNP MIXTURES, UP TO SIX CONTRIBUTORS

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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. From this panel we used 134 autosomal SNPs. Using the reference samples of three donors, we evaluated the strength of evidence with likelihood ratio (LR) calculations using the open-source quantitative model EuroForMix and compared the results with a previous study using a qualitative model LRmix. Both models were originally designed for multi-allelic STRs, but can be extended to bi-allelic SNPs. We showed that simple two-person mixtures can be readily analysed with both LRmix and EuroForMix, but the performance of three- or more person mixtures is generally inefficient with LRmix. Taking account of the read number (coverage) sequence information greatly improves the discrimination between true and non-contributors. The higher the mixture proportion (*Mx*) of the person of interest is, the higher the LR. Simulation experiments (up to six contributors) showed that the strength of the evidence is dependent upon *Mx* but relatively insensitive to the number of contributors. Hence estimating the number of contributors is not a limiting factor for the interpretation.

48. ESTABLISHING A RANGE OF FOUNDATIONAL VALIDITY FOR COMPLEX DNA MIXTURE INTERPRETATION USING PROBABILISTIC GENOTYPING SOFTWARE: A RESPONSE TO PCAST

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The President's Council of Advisors on Science and Technology (PCAST) 2016 report [1] on forensic science in criminal courts included criticism of the interpretation of complex DNA mixtures. The council noted that whilst "probabilistic genotyping software programs clearly represent a major improvement" in DNA interpretation, there is relatively little published in peer reviewed scientific journals about their validity for use with complex DNA mixtures. PCAST defines complex DNA mixtures as having low mixture proportions and high numbers of contributors, and urges the forensic community to collate and

publish validation studies that properly establish the range of reliability for these relatively new and promising approaches.
We present here the findings of a large inter-laboratory validation study, where ground truth known trials were conducted using the STRmix™ software on more than two thousand 3-to-5 person mixtures generated by over 30 laboratories, using a range of mixture proportions, multiplex, and CE platform protocols. The scope and limitations of the probabilistic genotyping software informed by this data are discussed, and evidence is provided that establishes a range of foundational validity for complex mixture interpretation using this method.

1. Executive Office of the President: President's Council of Advisors on Science and Technology. Report to the president Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods (2016).

49. IMPOSSIBLE TO SPECIFY THE NUMBER OF CONTRIBUTORS TO A DNA MIXTURE

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DNA·VIEW & Sr. Research Fellow UC Berkeley

Traditional advice about DNA mixture analysis says first decide the number of contributors. This advice is not only bad, it's impossible. It dates back twenty years to [1] -- but read the paper and you'll see it really meant "if the mixture is 2-person, here's how; if not, shelve it." Times have changed and now we do seriously consider complicated mixtures. Now is a good time to review and, without disrespect, to reject the old advice.
Sometimes there is no "the" number but rather prosecution and defense must choose different numbers. A mixture example will be presented for which the only reasonable prosecution hypothesis is a four person mixture, while the defense correctly sees it as three person. The resulting LR, for four person versus three, is close to neutral evidence. Calculating instead as four person throughout manufactures the illusion of strong evidence; it frames the suspect. Conversely, calculating uniformly as three person generates the illusion that the suspect is virtually excluded. Correctly employing the unconventional calculation makes a huge difference.
Examples for which the prosecution and defense must differ are moderately frequent in practice and demolish the rule to decide "the" number in advance. The examples do not follow a single pattern. A counterexample to a rule doesn't mean "better look out for this situation." It means the rule is wrong.

1. Clayton et al, Analysis and interpretation of mixed forensic stains using DNA STR profiling, For.Sci.Int 91 (1998) 55–70

50. SCIENCE ADVANCING JUSTICE: LESSONS LEARNED FROM DNA EXONERATIONS

Keynote Speech

S. Friedman

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The Innocence Project, founded in 1992 by Peter Neufeld and Barry Scheck at Cardozo School of Law,

exonerates the wrongly convicted through DNA testing and reforms the criminal justice system to prevent future injustices. An examination of the 350 DNA exonerations to date has identified five factors that contribute to wrongful convictions: eyewitness misidentification, misapplication of forensic science, false confessions, jailhouse informants, government misconduct, and ineffective defense counsel.
To protect the innocent, we work throughout the United States to ensure access to post-conviction DNA testing, eyewitness identification procedures are based on sound science, interrogations are recorded to reduce false confessions, and forensic disciplines used to identify suspects are valid and reliable.
Still we know the DNA exonerations are the tip of an iceberg since biological evidence is not available in most cases. The National Registry of Exonerations has documented an additional 1,704 non-DNA wrongful convictions. Getting it wrong harms more than an innocent person. There are families, crime victims and the public's confidence in the criminal justice system. Finally, there is a public safety concern as the actual perpetrator remains free to continue to commit crimes. Over the last 25 years, and in the next 25, the Innocence Project will continue to collaborate with a range of stakeholders to pursue reforms that ensure that the evidence used in the criminal justice system is accurate and reliable and thereby minimizes the chances of convicting an innocent person.

1. Brandon L. Garrett, Convicting the Innocent: Where Criminal Prosecutions Go Wrong, 2011; The Innocence Project. https://www.innocenceproject.org/

53. FIRST GENETIC EVIDENCE FOR JEWISH HOLOCAUST VICTIMS IN SOBIBOR, POLAND, BY PHYLOGEOGRAPHIC ANALYSES

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In the course of Europe's largest genocide of the past century victims of the German Nazi regime were systematically killed in extermination camps. One of these was located in Sobibor, in the eastern part of occupied Poland. This camp is known as the deadliest phase of the holocaust in German-occupied Poland with up to 200,000 people being murdered in gas chambers and subsequently cremated. After a revolt in 1943 the Nazis closed and completely destroyed the camp.
In 2014, a team of Polish archaeologists found remains of gas chambers in Sobibor. Surprisingly, also human remains were discovered, of which some showed signs of gunshot traces. According to historical data all Jewish victims of Sobibor were cremated. It was therefore hypothesized that the remains could belong to a group of resistants killed by the Polish communist government and secretly buried there in the 1950s.
The remains were subjected to molecular genetic testing in order to shed light into their possible ethnic background. This study included the analysis of entire mitochondrial genomes obtained from the remains by Massively Parallel Sequencing as well as Y-chromosomal markers, both of which are known to carry phylogeographic signatures. The resulting haplogroups were evaluated in the light of their distribution in present-day populations and were coinciding with lineages that are frequent in modern Ashkenazi Jews. To our knowledge this is the first attempt to use phylogeographic evidence to assign Holocaust victims to their respective source population

54. WHO'S THE DADDY? TRACING THE BREAK IN THE Y CHROMOSOME LINEAGE OF KING RICHARD III

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King Richard III, who was reported as being hunchback, was the last King of England to die in battle and one of the very few whose precise grave location had been lost in the over 500 years since his burial. In September 2012, excavations began to uncover any remnants of the Grey Friars friary, where Richard III was reported to have been buried following his death at the Battle of Bosworth in 1485. The remains of an individual: male, with battle injuries and scoliosis were uncovered during the course of this excavation and subsequent scientific analysis, including forensic, radiocarbon and mitochondrial DNA analysis of the remains, compared with two female-line relatives of the king, indicated that the evidence was overwhelming that these were the remains of Richard III1.
However, analysis of the Y chromosome revealed that there wasn't a DNA match between the skeletal remains and living male-line relatives, indicating that a false paternity/ies must have occurred in the 19 generations separating Richard III and the common ancestor of these living relatives. While unsurprising, we began a project to to narrow down the number of generations, or even pinpoint the exact generation, within which a false-paternity may have taken place.
Dr Turi King will discuss the strategies taken in tracking down where the break in the male line may have occurred, the present results, and next steps in this study which may shed light on the legitimacy on the historical monarchy of England in it's claims to the throne.

1. King, T.E. et al.: Identification of the remains of King Richard III, Nature Communications. 2014; doi: 10.1038/ncomms6631

55. WIDER USE OF DNA EVIDENCE IN CRIMINAL CASES: ANCESTRY PREDICTION USING Y CHROMOSOME ANALYSIS IN GERMANY

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In Germany an amendment of legislation concerning wider use of DNA evidence beyond identification is debated. The proposed change to law would allow analyzing the full DNA sequence checking for example hair-, eye color and biological age. Law enforcement, however, is pushing especially for inclusion of biogeographical ancestry prediction. Our laboratory has a 20 years experience in DNA-based prediction of ancestry for example in cases of unidentified persons. Currently, samples are analyzed using SNPs and STRs located on the Y chromosome, sometimes in combination with mtDNA. The eligibility of the Y chromosome for ancestry prediction is based on its unique power to differentiate between migratory events in human history. Descent clusters characterized by high frequencies of specific haplotypes and haplogroups are observed in certain geographical regions and estimates of the ages of such clusters are available due to intense research. The Y chromosome comprises slowly and rapidly mutating sites which can be used to analyze descent clusters in different time windows. Here we demonstrate our strategy for ancestry inference using different Y markers and population

databases. In an actual case we detected the founder mutation M198 (haplogroup R1a) in an unidentified corpse. This mutation occurred in Central Asia and subsequent migrations created descent clusters in Central-South Asia and Eastern Europe. The SNPs Z282 und Z93 differentiate the Asian and the European branch and could thus narrow down the regional origin to Eastern Slavic-speaking Europe. We discuss the prospects of this approach in the light of the current debate.

56. COMPARATIVE TOLERANCE OF TWO MASSIVELY PARALLEL SEQUENCING PLATFORMS TO COMMON PCR INHIBITORS FOR MISSING PERSON CASES

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Human remains are often challenging to identify as they may be highly degraded, fragmented, burnt, decomposed, or contain inhibitory substances. Massively parallel sequencing (MPS) has emerged as an alternative technology to current CE-based genotyping methods. The purpose of this study was to compare the inhibitor tolerance of two MPS-based systems specifically developed for forensic use and human identification (HID) purposes.
DNA (1 ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, and calcium). The samples were sequenced in triplicate using the HID-Ion AmpliSeq™ Identity Panel and Ion PGM™ (Thermo Fisher Scientific) in parallel with the ForenSeq™ DNA Signature Prep Kit (Primer Mix A) on the MiSeq FGx (Illumina).
Overall, each MPS system seemed to be more tolerant to some inhibitors than others. Both kits performed well with samples with very low levels of inhibitors. Both systems were tolerant to calcium; however, the AmpliSeq™ panel also performed well with collagen and melanin, but failed when even low levels of hematin were present. The ForenSeq™ kit performed relatively well with four inhibitors, but almost failed to genotype samples spiked with melanin.
This study also examined the relative effectiveness of the most commonly employed DNA extraction protocols used in forensic laboratories to extract high levels of these five inhibitors from blood, hair, muscle, and bone samples prior to genotyping. These results demonstrate the most compatible sample preparation methods for highly inhibited biological samples on the Ion S5 and MiSeq FGx systems.

57. LESSONS FROM A STUDY OF DNA CONTAMINATIONS FROM POLICE SERVICES AND FORENSIC LABORATORIES IN SWITZERLAND

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In Switzerland, the DNA profiles of police officers collecting crime scene samples as well as forensic genetic laboratories employees are stored in the staff index of the national DNA database to detect potential contaminations. Our study aimed at making a national inventory of contaminations to better understand their origin and to make recommendations in order to decrease their occurrences. For this

purpose, a retrospective questionnaire was sent to both police services and forensic genetic laboratories for each cases detected.
Between 2011 and 2015, a total of 709 contaminations were detected. This represented a mean of 11.5 (9.6 – 13.4) contaminations per year per 1'000 profiles sent to the Swiss DNA database. Answers to the questionnaire were obtained from the police, the laboratory or both for 552/709 (78%) of the contaminations. About 88% of these contaminations were from police officers whereas only 12% were from genetic laboratories employees. Interestingly, there has been a direct contact between the stain and the contaminant person in only 51% of the laboratory contaminations whereas this number increases to 91% for police collaborators. The high level of indirect DNA transfer in laboratories might be explained by the occurrence of "DNA reservoirs" suggesting that cleaning procedures should be improved in laboratories. At the police level, most contaminations were from the person who collected the sample and likely occurred on the crime scene. This suggests that improving sampling practices (e.g. increasing the frequency of glove changes and systematically wearing masks) could be beneficial.

58. DEGRADED DNA SAMPLES MADE INFORMATIVE BY USING SUPERPRIMERS

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Available commercial Short Tandem Repeats (STRs) multiplex kits are a reliable and widespread resource for obtaining individual genetic profiles from several types of DNA samples[1,2,3]. However, they are not well suited for fully amplifying the degraded DNA found in most archaeological, aged and forensic samples[4]. This shortfall arises from the need to amplify non-specific sequences[5] in order to obtain large amplicons that are suitable for capillary electrophoresis (CE) size discrimination. That may be overcome by using long ssDNA polynucleotides as primers[6], then greatly improving the detection of genetic markers in degraded DNA. Long primers of up to 200 nucleotides (a.k.a. *superprimers*) allow a closer annealing to the target repeat sequences, thereby reducing the length of the intact DNA required for polymerization, while at the same time rendering amplicons of large size suitable for CE detection in multiplex assays. S*uperprimers* can be designed to anneal adjacently to the target repeat regions, then amplifying fragmented DNA in a similar fashion to the mini-STRs[7]. We have also demonstrated that *superprimer* annealing is sequence-flexible (*i.e.* non-fully complementary), thus allowing for the design of practically any primer sequence. We show hereby that the use of long ssDNA polynucleotides on degraded cadaveric DNA samples can discriminate genotypes otherwise missed by the currently used commercial kits.

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3. Mullis KB: Process for amplifying nucleic acid sequences. US Patent No. 4,683,202. Emeryville, 1987.
4. Butler JM: Forensic DNA Typing, Biology, Technology and Genetics of STR Markers,2nd Ed. Burlington: Elsevier Academic Press; 2005.
5. NIST Short Tandem Repeat DNA Internet Databa: http://www.cstl.nist.gov/strbase/index.htm

59. DNA TYPING FROM SKELETAL REMAINS: A STUDY OF INHIBITORS USING MASS SPECTROMETRY

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This project examines the materials co-extracting with DNA from skeletonized remains that have been in the environment for greater than 50 years. A total of 435 samples with known loss locations were collected for this study in the course of ongoing HID processes. During preparation for DNA extraction, a fine powder containing osseous materials and associated environmental detritus was collected from these skeletal elements. Portions of this skeletal residue were extracted with multiple solvents and evaporated to concentrate available materials. Samples were rehydrated in methanol and analyzed using a GC/MS and/or an LC/MS-MS. Additionally, the purified DNA from the associated remains was suspended in methanol for comparison. Initial results indicate that accelerants and other fuels are not completely removed from DNA extracts using an organic extraction protocol. The skeletal materials were a mix of materials present in the environment, by-products of decay (e.g., lipids), and fat-soluble compounds inherent to the remains. Fat-soluble medications (e.g., quinine) were detectable, as were fuels and accelerants. Site-specific biological materials, such as oils from local plants, were also detected. Comparison of skeletal elements from the same site, but not the same individual, showed similar patterns of compounds present with personal variations. Not only is it possible to qualitatively study the presence of DNA inhibitors in real-world situations using MS, but there is the potential to provide an additional metric for individuation or identification of unknown human remains.

60. KPOP: A PYTHON PACKAGE FOR POPULATION GENETICS ANALYSIS

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Kpop is an open source Python package that detects population structure from biallelic data. It implements its own maximum-likelihood routine to estimate admixture coefficients and provides an interface to run Structure [1], ADMIXTURE [2] and Arlequin [3]. Kpop also simulates population dynamics, including a few different models for hybridization and genetic drift. This unified framework makes it convenient to assess how different histories of hybridization and isolation can produce certain patterns of population structure. Kpop integrates Scikit-Learn [4], and Tensorflow [5] packages that provide state-of-the-art implementations of machine learning (ML) algorithms in Python. While some techniques are widely used by the forensic genetics community (e.g., PCA, for dimensionality reduction), other techniques with similar objectives are not so common (e.g., t-distributed Stochastic Neighbor Embedding [6]).
Kpop integrates algorithms for many common Machine learning fields that are useful for processing genetic data: dimensionality reduction, hard and fuzzy clustering, classification, preprocessing of datasets, etc.
This paper introduces Kpop's main functionalities and compares standard methods for dimensionality reduction and clustering of genetic data with some alternatives used by the machine learning community. In many cases, the ML counterparts provide superior performance on difficult to handle

datasets.

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61. A LARGE SCALE STUDY ON THE CHARACTERISATION OF DROP-IN WITHIN A DNA LABORATORY

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Forensic DNA analysis has become increasingly sensitive in recent years with the advent of improved PCR chemistries and highly sensitive CE instruments. This has allowed the detection of STR alleles from single copies of input DNA. As a consequence of this increased sensitivity and increases in the number of loci tested; understanding and characterising drop-in events become of greater importance. Drop-in is defined by the DNA commission of the International Society of Forensic Genetics as; one or two alleles that “come from *different* individuals” [1]. Many models have been developed to describe drop-in that can be applied to a continuous probability. However, no large study of drop-in has been published within the literature.

Here we present a review of 13,485 negative control samples processed over a period of 18 months (April 2015 – September 2016). In this review multiple characteristics of drop-in are assessed including drop-in rates, peak heights, locus trends and allele frequencies. Two additional sets of experimental data have been generated for comparison to this data set. These consist of: 1) Experimental ESI 17 Fast data on very low DNA inputs (0.01pg - 20pg); 2) In silico modelling of low-level sampling variation using a Poisson distribution.

These data highlight that the distribution of drop-in alleles is consistent with population allele frequencies and that drop-in rates vary over time, even within the same DNA laboratory. Our experimental data also demonstrate that very low level genomic DNA can result in profiles with just one or two allele peaks which are indistinguishable from drop-in.

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62.PROTEIN-BASED FORENSIC IDENTIFICATION USING HAIR SHAFT IN EAST ASIAN AND EURASIAN POPULATIONS

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The development of forensic DNA methods have advanced rapidly over the past few years, especially short tandem repeats (STRs) genotyping for individual identification and single nucleotide polymorphisms (SNPs) genotyping for biogeographic inference. All these analyses depend on the nuclear DNA and PCR, however, hair shaft is poor source of nuclear DNA template due to keratinocyte apoptosis. One latest research demonstrated that identity discrimination can be obtained from detecting genetically variant peptides (GVP) in hair shaft protein [1].
In total we collected hair shaft samples of 100 Han Chinese (East Asian) and 100 Uyghur (Eurasian) individuals. According to the paper's method, we have established the hair shaft proteomics purification procedure, and analyzed mass spectrometry-based shotgun proteomics in 30 individuals. Using the paper's custom reference protein database, 181 SAPs were found from 212 GVPs in105 genes, and the frequency of 7 SAPs loci is significantly different in Han Chinese and Uyghur subjects. Our ongoing work is to analyze SAPs in all 200 individuals and infer the non-synonymous SNPs, then evaluate the performance of hair shaft in individual identification and biogeographic inference.

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63. BEYOND THE STRS: A COMPREHENSIVE VIEW OF CURRENT FORENSIC DNA MARKERS CHARACTERIZED IN THE PCR-BASED DNA PROFILING STANDARD (SRM 2391D)

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Capillary electrophoresis (CE) of the COmbined DNA Index System (CODIS) core set of 20 Short Tandem Repeat (STR) markers [1] is the primary genotyping method for forensic human identification in the U.S. With the emergence of new technologies beyond CE testing, other categories of forensic DNA markers are now more readily available. The next iteration of the PCR-Based DNA Profiling Standard Reference Material, SRM 2391d, will contain allele calls and sequence information for forensically relevant markers available in commercial kits. The SRM components will be characterized using CE and next generation sequencing (NGS). With NGS technology, more data can be mined from these reference samples than previous generations of this SRM2. This presentation will focus on data from the forensic DNA markers beyond the common autosomal, Y-STR, and X-STR markers to include: insertion/deletions, ancestry single nucleotide polymorphisms (SNPs), identity SNPs, phenotypic SNPs, microhaplotypes and mitochondrial whole genome sequencing. The primary emphasis will be an analysis of the information gained from using NGS vs CE technology with the reference samples going "Beyond the STRs".

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64. INTER-LABORATORY VALIDATION STUDY OF THE FORENSEQ DNA SIGNATURE PREP KIT, MISEQ FGX INSTRUMENT, AND FORENSEQ UNIVERSAL ANALYSIS SOFTWARE FOR LENGTH-BASED STR ANALYSIS

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The ForenSeq DNA Signature Prep kit enables the simultaneous library preparation and massively parallel sequencing of 58 STRs and up to 172 SNPs. The abundance of sequence data generated from a single reaction reduces sample consumption (5 µL volume input) while maximizing genetic marker throughput and power of discrimination. To demonstrate the validity of this kit for the U.S. National DNA Index System, an inter-laboratory validation study was performed at three U.S. laboratories. This collaborative project assessed sensitivity, repeatability and reproducibility, mixtures, non-probative mock samples, and contamination using both DNA primer mixes (DPMA and DPMB). The manufacturer's protocol was followed for processing 32 samples simultaneously, and analysis was performed in the Universal Analysis Software. The testing involved over 1500 samples that were evaluated across 49 sequencing runs on five MiSeq FGx instruments. As a first step to implementing this method, analysis focused strictly on length-based STR calls to maintain backwards compatibility with current databases. Across laboratories and instruments, numerous control DNA and reference sample genotypes were shown to be highly accurate and reproducible. Full STR profiles were observed from DNA inputs down to 63 pg for DPMA and 125 pg for DPMB, and minor allele detection averaged 74% in 1:19 mixture ratios. Non-probative samples resulted in over 99% concordance with CE data. The loci D22S1045 and DYS392 notably underperformed throughout the study. This inter-laboratory validation confirmed the overall robustness of ForenSeq for length-based STR analysis and its utility with current procedures.
The opinions or assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the U.S. Army Medical Research and Materiel Command, the Defense Health Agency, or the Armed Forces Medical Examiner System.

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266	Emel Hülya Yükseloğlu	Identification of oral swab samples with the latest DNA technology (24 STR DNA loci): A validation study
267	Martha Lucia Camargo	Magnetic beads: An alternative method to enzymatic purification for SNaPshot reactions
268	Daniel Corach	Measuring human DNA degradation and gender detection in forensic DNA samples by q-PCR / HRM analysis
269	Sheri J Olson	Methods for improvement of allele recovery with the GlobalFiler assay
270	Bruce Budowle	Micro sample swabbing for reduced sample consumption, increased sensitivity of detection and enhanced intelligence for processing biological evidence
271	Sehee Oh	PCR-reverse blot hybridization assay for Y haplogroup prediction
272	Veena ben Trivedi	Performance comparison of two most recent Y-STR multiplex systems for real forensic casework analysis
273	Sara Palomo-Díez	Prep-n-Go™: A new and fast extraction method for forensic blood samples
274	Ju Yeon Jung	Re-establishment of the VNTR D1S80 analysis system and new method for allelic ladder construction
275	Rashed Alghafri	Reduced volume for direct PCR amplification of blood reference samples using Identifiler® Direct and Globalfiler™ Express
276	Maryam Sharafi Farzad	Reduced volume PCR amplification using the AmpFlSTR® NGM SElect™ PCR Amplification Kit for stain samples
277	Sara Palomo-Díez	Sex molecular diagnosis on critical samples: Comparison of different methodologies
278	Mechthild Prinz	Simultaneous DNA and protein extraction using trypsin
279	Shota Inokuchi	Simultaneous human identification and animal species discrimination employing developed blocking-PCR from human/animal mixture sample
280	Weibo Liang	SNP-STR analysis for noninvasive paternity test of fetus
281	Sun Seong Choi	Target sequencing panel for Korean-specific autosomal SNPs
282	Selina Burch	The Applied Biosystems™ NGM Detect™ PCR Amplification kit - As promising as promised?

283	JeongEun Sim	The development and validation of homemade STR PCR kit composed of expanded 20 autosomal loci
284	Vladimir Aleksandrovich Orekhov	The duplication of vWA allele may cause discordant STR profiles
285	Vanessa Bogas	The effect of different levels of degradation and DNA concentrations on the quality of genetic profiles
286	Laura Pelleymounter	The GlobalFiler kit system: Enabling greater flexibility-Responses to feedback from laboratories
287	Gesine Susann Schwerdtner	The separation of male and female: A comparison of seven protocols
288	Goli Fahid	Updated AutoMate Express™ Extraction System Protocol Card with variable elution volumes
289	Dragana Zgonjanin	Validation and implementation of the Investigator® 24plex QS kit for forensic casework
290	Sasitaran Iyavoo	Validation of ABI 3500xL Genetic Analyzer after decommissioned and recommissioned at new premises
291	Vanessa Bogas	Validation of Sampletype I-sep DL for differential extraction and purification with Prepfiler Express in the AutoMate Express DNA Extraction System
292	Samara Garrett-Rickman	Validation of the SureID® Compass Human DNA Identification Kit
293	Nicoletta Cerri	Validation studies and forensic applications of Investigator 24plex QS Kit
294	JiYoung Kim	The study of ABO genotyping for forensic appilication

TOPIC 05 — Body Fluids Identification, Touch DNA, Forensic Biology, Legal Medicine

295	Carlo Robino	A GEFI collaborative exercise on DNA/RNA co-analysis and mRNA profiling interpretation
296	Sharon Elizabeth Doole	An assessment of the ParaDNA body fluid ID system in a casework environment
297	Chong Min Choung	Application reports of Body fluid identification by the DNA methylation
298	Eirik Natås Hanssen	Body fluid prediction from microbial patterns for forensic application
299	Myung Jin Park	Casework of vaginal fluid identification using DNA methylation profiling
300	Maria Inês Silva Gregório Martins	Characterizing sanitary napkins and diaper types as crucial evidences for semen identification
301	Cornelius Courts	Detection and identification of forensically relevant organ tissue types and skin by micro-RNA expression analysis
302	Laura Dodd	Developmental validation of the ParaDNA Body Fluid ID System
303	Erhan Acar	Development of a multiplex mRNA panel for identification of

		body fluids
304	Nunzianda Frascione	Development of biosensors for forensic analysis
305	Shuntaro Fujimoto	Effect of the absence of spermatozoa on microRNA-based semen identification
306	Olatunde Abimbola Afolabi	Evaluation of genetic markers for forensic identification of human body fluids
307	Han-Chul Lee	Evaluation of the BMS® Rapid Stain Identification Multiplex Kit for use in body-fluid identification
308	Christophe A Marquette	Evaluation of the Sperm Tracker™ for semen stains localization on fabrics
309	Peng Bai	Expression changes of microRNAs in menstrual blood samples of different menstrual cycle collection days
310	Weibo Liang	Expression difference of miR-10b and miR-135b between the fertile and infertile semen samples
311	Carlo Robino	Finding optimal sample collection strategies for DNA/RNA co-analysis of forensic stains
312	Yiping Hou	Microarray expression profile of circular RNAs in human body fluids
313	Matteo Fabbri	mRNA profiling in ancient bloodstains
314	Matteo Fabbri	mRNA profiling: Application to an old casework
315	Yuchih Lin	Novel identification of biofluids using a multiplex methylation sensitive restriction enzyme-PCR system
316	Cornelius Courts	Organ tissue identification by micro-RNA expression analysis in realistic forensic type samples
317	Suaad Alshehhi	Quantification of RNA degradation of blood-specific markers to indicate the age of bloodstains
318	Tomoko Akutsu	Quantitative evaluation of candidate genes and development of a multiplex RT-PCR assay for the forensic identification of vaginal fluid
319	Peng Bai	Quantitative method - A crucial choice on forensic body fluids identification using miRNAs
320	Thomas Berg	Sensitivity of semen identification tests. A study on biological stains exposed to outdoor conditions for up to 365 days
321	Dayse Aparecida Silva	SERATEC - Study on the sensitivity and specificity of current presumptive methods of semen detection in forensic samples
322	Jee Won Lee	Simple and rapid identification of oral Streptococci in saliva by immunochromatographic strip
323	Chong Wang	Skin contact stains identification based on mRNA profiling
324	Maria Inês Silva Gregório Martins	Superabsorbent lower-layers of sanitary pads: A challenge in sexual aggression cases
325	Galina Kulstein	The changing face of body fluid identification: Can molecular m(i)RNA-based methods outdo conventional approaches for the identification of laundered seminal stains?

326	James Gooch	The development of aptamers for body fluid identification
327	Rachel Fleming	To move or not to move - Massively parallel sequencing vs RT-PCR for body fluid identification
328	Jessica Teschner	TSMP - STR profile and body fluid identification in one analysis
329	Ane Elida Fonneløp	A Bayesian network to evaluate DNA transfer in an attack scenario
330	Sophie Mary Park	A comparison of minitaping and swabbing for collecting DNA from non-porous surfaces
331	Shengting Li	An evaluation of the performance of DNA recovery from fired firearms and cartridge cases using microdialysis filtration
332	Jennifer EL Templeton	Analysis of STR-based DNA profiles from complex substrates
333	Peng Bai	Comparative study on methods of DNA extraction and genotyping between single piece of dandruff and EZ-tape
334	Phuvadol Thanakiatkrai	Direct STR typing from bullet casings
335	Jakub Czarny	DNA Tape for studies on sexual offences
336	Hisanori Muramatsu	DNA testing of a trace bloodstain on a laundered jacket
337	Roland A.H. Van Oorschot	DNA transfer by different parts of a hand
338	Dayse Aparecida Silva	Efficiency of DNA recovery from fingerprints enhanced with black and magnetic powders
339	GANGNAM JIN	Evaluation of M-VAC® system for collection of trace DNA from mock samples
341	Roland A.H. Van Oorschot	Hand activities during robberies - Relevance to consideration of DNA transfer and detection
342	Dina Aloraer	Improving touch DNA recovery
343	Mark Barash	Laundry in a washing machine as a mediator of secondary and tertiary DNA transfer
344	Karl Reich	Materials and methods that allow fingerprint analysis and DNA profiling from the same latent evidence
345	Ann Marie MacPhetridge	Normalization and direct amplification of casework samples
346	Céline Maria Pfeifer	Persistence of wearer DNA on clothing
347	Toni Boyko	Prevalence of DNA from driver, passenger and others within a car of an exclusive driver
348	Georgina Emma Meakin	Recovery of DNA from regularly-worn hoodies: Consideration of recovery method and sampling location
349	Dusan Keckarevic	Success in obtaining interpretable DNA profile from cartridge casings using different methods of extraction and DNA amplification kits - comparative study
350	Georgina Emma Meakin	The effect of pressure on DNA deposition by touch
351	Georgina Emma Meakin	The effects of recovery method and sampling location on DNA from regularly-worn hoodies subsequently worn by a different individual

352	Larissa Barbosa Nunes	The influence of fingerprint powerds in the genetic profiles
353	Thitika Kitpipit	Touch DNA localization and direct PCR: An improved workflow for STR typing from improvised explosive devices
354	Eva Schultheiss	Transfer and persistence of DNA as a function of the shedder status
355	Roland A.H. Van Oorschot	Transfer of picked-up DNA to cotton plates
356	Jennifer EL Templeton	Typing DNA profiles from previously enhanced fingerprints using direct PCR
357	Ugo Ricci	Who touched that woman? Opportunity and limits of touch DNA
358	Nicoletta Cerri	A comparative study of human and animal hairs: Microscopic and Cytochrome Oxidase I (COI) species identification
359	HEE-SOO KIM	A preliminary study on the NGS analysis of the microbial community structure in the drowned pig's skin
360	Daniel Vanek	A validation study for the extraction of DNA from forensic samples using MagCore HF 16 Plus automated nucleic acid extractor
361	Dayse Aparecida Silva	Analysis of human DNA present in the digestive tract of *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes for possible forensic application
362	Baek Yoon Gi	Analysis of marine microorganisms extracted from the internal organs of a drowned pig
363	Man Il Kim	Aptamer-based sensor for the identification of female-specific blood stain
364	Martha Lucia Camargo	Assesment of method for buccal swab samples preservation in extreme environmental conditions for population genetics and forensic purposes
365	Vanessa Bogas	Comparative study between a direct DNA quantification methodology and the standardized methodology in the forensic workflow
366	Marie-Louise Kampmann	Decrease DNA contamination in the laboratories
367	Peng Bai	Degradation of AIF mRNA in rat heart tissue for estimating postmortem interval (PMI)
368	Alicia Haines	Detection of DNA present on tape-lifts using fluorescent *in situ* dectection
369	Andrew Schlenker	Development of a triaging process using Quantifiler HP to assist in choosing an appropriate downstream amplification method for post mortem identification of human remains
370	JEONG CHAN MOON	DNA analysis of drug-related crimes occurred in Gyeongsangnam-do and Busan in 2016
371	Sirirat Mienkerd	DNA examination of sexual assault with multiple assailants
372	Sang Eon Shin	Establishment of basic forensic entomological data for forensic application
374	Kevin WP Miller	FlipTubes® technology promotes clean manipulation of forensic samples on automated robotic workstations

375	Ji Zhang	Forensic drowning site identification employing a mixed pyrosequencing profile of diatom DNA-barcode
376	Jakub Czarny	Forensic identification of soil using metapopulational analysis of the bacterial IV 16SRNA region
377	Xiling Liu	Genome-wide copy number variation analysis in monozygotic twins
378	Fanming Meng	Influence of insect activity on the dynamic change of postmortem microbial communities
379	Yueh shyang Ping	Modified differential DNA extraction to reduce processing time of sexual assault exhibits
380	Karl Reich	PCR-based tests for forensic detection of feces; Use of bacteroides species as indicators of fecal contamination
381	Lin Zhang	Postmortem interval (PMI) determination by profiling of haf mRNA degradation using RT-qPCR
382	Sara Palomo-Díez	Presumptive tests: A substitute for Benzidine in blood samples recognition
383	YaDong Guo	The effects of methamphetamine on the development of blowfly *Aldrichina grahami* (Diptera: Calliphoridae) and cadavers bacterial community
384	Melissa R Schwandt	The use of Whatman™ FTA™ Elute to simplify storage and reanalysis of extracted DNA from forensic samples
385	PRAPAPORN KHAIPRAPAI	Using DNA for drug smugglers
386	Suthamas Phuengmongkolchaikij	Alcohols as solution for delaying microbial degradation of biological evidence on cotton swabs
387	Ukhee Chung	Analysis of mixture DNA in murder case evidences
388	Martha Lucia Camargo	Case report: Male phenotype with incomplete Y chromosome and X chromosome double dosage
389	Kornkiat Vongpaisrnsin	Circulatory microRNA in acute myocardial infarction: A candidate biomarker for forensic investigation
390	Daniel Vanek	Comparison of fluorometric and real-time PCR quantification of DNA extracted from formalin fixed tissue
391	Henrik Green	Genetic screening of sudden cardiac death genes in ffpe samples using haloplex hs
392	Korapin Srisiri	Optimisation of DNA recovery and storage for urineauthen-ication

TOPIC 06 Ethics & Legal

393	Dayse Aparecida Silva	A mini-primer set in a multiplex PCR fashion covering the mtDNA control region from skeletal remains submerged
394	Christian Gehrig	A swiss collaborative simulated exercise for DVI: Lessons learned using the familias software
395	Amy Sorensen	Alternative methods for collection, room temperature storage, and processing of DNA samples from human remains: A new DVI approach

396	Jung-Hyun Park	Autosomal single nucleotide polymorphisms-based inference of relatedness for identification of missing casualties
397	Jodie Ward	Best practice recommendations for the establishment of a national DNA identification program for missing persons: A global perspective
398	Dragana Zgonjanin	DNA analysis from human skeletal remains in forensic casework
399	Lais Vicente Baptista	DNA persistance in soft tissue using vodka as preservative agent
400	Wiliam H Goodwin	DNA-assisted identification in post-conflict environments
401	Sheree Hughes-Stamm	Do we really need to crush? An alternate DNA extraction approach for bone samples.
402	Sasitaran Iyavoo	Evaluation of decalcification for recovery of DNA from bone
403	Zhong Wu	Forensic performance of a very large human identity SNP panel.
404	YUNA OH	Genetic analysis of old skeletal remains from Korean War victims using PowerPlex® Fusion 6C and miniSTR system for human identification
405	Andrzej Ossowski	Massive identification of victims of Totalitarian systems and Holocaust in Poland
406	Frank Götz	Missing person and DVI with cloud based software
407	Anne Stone	Recovery of exomes and mitochondrial genomes from dental calculus
408	Andrea Carbonaro	What we can learn from ancient bones - Y-STR analysis of human skeletal remains from the American Civil War, World War II, Seven Years' War, and the American Old West
409	Irina Olegovna Perepechina	Body fluid stain as a forensic object: In the shadow of enormous achievements in DNA analysis other essential aspects of the examination can be eclipsed
410	Helena Cristina Machado	Challenges of communicating forensic genetics to multiple publics
411	Kyu Won Jung	How does forensic science fit into Korean society?
412	Irina Olegovna Perepechina	On the standard of the genetic identity determination on the basis of the consensual level of reliability of DNA identification
413	Nicoletta Cerri	Young people's perspective on the Italian forensic DNA database: Results of a pilot study on university students
414	Si-keun Lim	Challenges and chances of forensic DNA analysis and DNA database in Korea
415	Taehee Um	DNA database operation and hit cases in the Korean Prosecution
416	Thitima Sanpachudayan	DNA profiling database: Fungi attack
417	Nongnuch Boonderm	Effectiveness of CIFS DNA database in Thailand
418	Andreas Tillmar	Increasing the evidential weight in relationship testing by taking STR DNA sequence polymorphisms into account

419	Ugo Ricci	Moving towards a shared forensic standard in Italy
420	Mannis van Oven	PhyloTree Build 18: Update of the human mtDNA phylogeny
421	Ines Pickrahn	Police officers DNA on crime scene samples - Indirect transfer as a source of contamination and its database-assisted detection in Austria
422	Nicole Huber	Search, align and haplogroup - Improved forensic mtDNA analysis via EMPOP
423	Meiga Aurea Menezes	The Brazilian DNA database: A view after 04 years
424	Zlatko Jakjovski	The power of forensic DNA data bases in solving crime cases

TOPIC 07 — Predictive Markers

425	Ji Zhang	A short unix shell script for VCFtools commands iteration to obtain the genetic characteristics of variants for forensic purpose
426	Irina Olegovna Perepechina	Adoption of the decision concerning genetic identity in the framework the decision theory: Verification of the model parameters by empirical data
427	Eugenio Alladio	Challenges in LT-DNA mixtures interpretation: A proof-of-concept multi-kits, multi-instrument and multi-software comparison when approaching highly challenging samples
428	Øyvind Bleka	DNAmatch2: An open source software for carrying out large scale contamination searches using qualitative and quantitative models
430	Tatiana Hessab	Evaluating the performance of forensic genetics statistics in Brazilian population
431	Xudong Wang	Evaluation of the full-sibling kinship with multiple full-siblings attendance
432	Tatiana Hessab	False inclusion risk of duo parentage cases
433	Peter Gustav Ristow	Forensic statistics ananlysis toolbox (forstat): A streamlined workflow for forensic statistics
435	Frank Götz	New software and process to resolve complex DNA mixtures
436	Daniel Kling	SNP analyzer - A tool to analyze large sets of genetic markers accounting for linkage
437	JIEUN PARK	Statistical estimation of ambient temperature for minimum Post-Mortem Interval(PMI) in Korea
438	Leonor Gusmão	The influence of the different mutation models in kinship evaluation
439	Meng-Han Lin	The interpretation of DNA mixtures with the presence of relatives
440	Nor Aidora binti Saedon	The significance of statistical probability for loci above 20
441	Daniel Kling	Unexpected effects of mutations in kinship testing
442	Hussain Ahmed Alsaleh	A multi-tissue age prediction model based on DNA

		methylation analysis
443	Toshimichi Yamamoto	A personal identification trial with post-sucking time estimation from human blood in mosquitoes
445	Yiping Hou	Age-associated DNA methylation determination of semen by pyrosequencing in Chinese Han population
446	Hyun-Chul Park	An integrated analysis tools for CpG island prediction and primer design
447	Michelle Spiden	Assigning phenotypic and ancestry information to Victoria's unidentified human remains - A pilot study
448	Yong Han Phua	Autosomal and Y-STR: A combinatory approach to ethnicity inference in Singapore
449	Eun Hee Lee	BMI-associated SNP genotypes and body shape prediction in a Korean population
450	Christopher Phillips	Casework experiences with detailed SNP-based ancestry analyses using Ion Torrent massively parallel sequencing
451	Yiping Hou	Characteristics of SNPs related with high myopia traits in Chinese Han population
452	Vania Pereira	Comparison of ancestry estimation strategies using different marker sets
453	Anastasia Paraskevi Aliferi	Correlating DNA methylation data with chronological age: A quest for the optimal statistical model
454	Christopher Phillips	Development of a new age prediction assay using the MS-SNuPe strategy for forensic purposes
455	Christopher Phillips	Evaluation of Clock genes expression patterns for prediction of the time-of-deposition of a biological sample
456	Zhonghui Thong	Evaluation of DNA methylation-based age prediction on bloodstain samples
457	Christopher Phillips	Forensic age estimation tests extended to DNA methylation patterns in children and adolescents
458	Christopher Phillips	Forensic age prediction models: Adapting DNA methylation data from pyrosequencing tests to a quantile regression model
459	Lin Zhang	Genotyping of eleven SNPs associated with facial hair in Chinese Han population
460	Kiros Haddish	Helping the identification of refugee shipwreck victims in the Straits of Sicily: An AIM-Indel reference database for the Tigray population of Ethiopia
461	Jan Felix Fleckhaus	Impact of genetic ancestry on chronological age prediction using DNA methylation analysis
462	Magdalena Kukla-Bartoszek	Improving prediction of pigmentation traits by quantitative phenotype evaluation and massively parallel sequencing
463	Amke Caliebe	Likelihood ratio and posterior odds in forensic genetics: Two sides of the same coin - With application to forensic DNA phenotyping
464	Fan Liu	Meta-analysis of genome-wide association studies identify 9 novel loci involved in human hair curliness

465	Bailey Harrington	Morphometrics, craniofacial disease genes, and the quest for the genetic basis of facial morphology
466	Sara Palomo-Díez	Phenotyping the ancient world: The physical appearance and ancestry of very degraded samples from a Chalcolithic human remains
467	Jong-Lyul Park	Potential forensic application of DNA methylation profiling for identical twin identification
468	Elaine Cheung	Prediction of admixed biogeographical ancestry
469	Helle Smidt Mogensen	Prediction of biogeographical ancestry by a hypothesis testing approach with genogeographer
470	Mark Barash	Prediction of externally visible characteristics of a person from a DNA sample using deep machine learning approach
471	Jakub Czarny	Predictive DNA analysis by on-array minisequencing in DNA imaging technology
472	Anna Woźniak	Premature hair greying in young people and age associated DNA methylation differences based on 5 age related markers
473	Jeppe Dyrberg Andersen	Sequencing of the HERC2-OCA2 region in relation to human eye colours
474	Weibo Liang	Single/double eyelids associated genes in eastern
475	Yi Ye	The confirmation of genetic variants associated with the subjective response after alcohol consumption
476	Jan Felix Fleckhaus	The impact of different carrier materials on the stability of DNA methylation levels for age prediction after long term storage of human blood stains
477	Chong Min Choung	The usefulness of the age prediction method in forensic DNA samples
478	Ewelina Pośpiech	Unraveling the complex genetics of human head hair features to facilitate their prediction accuracy in forensics
479	Ali Miri	Victim identification by genetic analysis in a human disaster
480	Yahya Khubrani	Extensive population structure in the Y-chromosome landscape of Saudi Arabia revealed by analysis of 27 Y-STRs
481	Craig Nolde	Dramatically increasing throughput for DNA Y-Screening of sexual assault evidence by 96-well plate processing
482	Valeria Cecilia Marcucci	A compromising position for a variant: A new variant of D1S1656 that invades its neighbors and can lead to missinterpretation

Topic 01: Massively Parallel Sequencing

1. A COMPARISON OF WORKFLOW AND PERFORMANCE BETWEEN MASSIVELY PARALLEL SEQUENCING AND CAPILLARY ELECTROPHORESIS APPROACHES FOR FORENSIC STR GENOTYPING

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Currently, PCR and capillary electrophoresis (CE) coupled platforms are routinely used by most forensic DNA laboratories despite limitations such as throughput, the number of markers which can be genotyped and inability to detect intra-locus polymorphisms. Massively parallel sequencing (MPS) approach overcomes most of these limitations enabling sequencing hundreds of genetic markers at single nucleotide resolution in a time– and cost– efficient manner.
This study compared MPS and CE approaches in regards to their workflow, performance and resolution, utilizing an Early Access (EA) STR MPS panel and a GlobalFilerTM CE kit (both by Thermo Fisher Scientific). The MPS panel performance was tested with 96 DNA samples of various quantity and quality, including mixed source samples. Manual and automated workflows were assessed for library preparation. A subset of samples (n=48) was processed manually using a CE-coupled kit. The metrics that were evaluated included the time and labour required for sample processing, reliability, concordance, sensitivity, robustness, as well as mixtures and kinship analyses.
The EA STR MPS panel produced reliable results, generating full profiles from a DNA template inputs ranging between 1ng and 125pg and partial profiles from as low as 31pg. The MPS data was concordant with the CE-generated profiles for pristine and challenging samples. With continuous research and technical advances in MPS, it has the potential to become a routine method of human genotyping in forensic DNA laboratories. This work demonstrates an important milestone leading towards this goal.

2. A STUDY OF DEGRADED BONE SAMPLES USING CE AND NGS METHODS

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DNA analysis from human remains is of immense relevance in missing persons identification and disaster victim identification (DVI). DNA degrades gradually in hard tissues, such as bones and teeth under a high temperature, humidity, pH, geochemical properties of the soil, the presence of

microorganisms and all other factors that affect the preservation of DNA in skeletal remains. Recent advances in massively parallel sequencing (MPS, provides some advantages over previously used technologies to analyse DNA from human remains, particularly ancient. The aim of this study was to investigate if we could use the Illumina ForenSeq DNA signature kit to genotype autosomalSTRs, X and Y STRs as well as SNPs in bone samples for use in disaster victim identification. The beta-version of the ForenSeq kit was used to genotype 32 bone samples from Serbia lab that previously profiled using GlobalFiler amplification kit using 3500 capillary electrophoresis (CE). A total number of 86 samples were typed on 3500 CE and 32 were selected for NGS work. Results of Run on FGx-MiSeq sequencing showed a cluster density of 866 K/mm2. A total 95% of clusters generated run passed filters. The results of the samples will be shown between the CE and NGS results. The beta-test worked rather well for a beta-test using one independent run. Ancestry, Identity and phenotypic SNPs had been typed in the degraded samples. The CE GlobalFiler data -STR markers and the Illumina ForenSeq DNA signature kit showed remarkable results for concordance.

3. ANCESTRY ANALYSIS OF MAINLAND JAPANESE AND OKINAWA JAPANESE POPULATIONS USING THE PRECISION ID ANCESTRY PANEL

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We typed 165 AIMs in 49 mainland Japanese and 47 Okinawa Japanese using the Precision ID Ancestry Panel (Thermo Fisher Scientific). None of the 165 SNPs were in Hardy-Weinberg disequilibrium in the mainland Japanese. One SNP (rs3943253) showed Hardy-Weinberg disequilibrium in Okinawa Japanese. Fisher's exact tests showed that the genotype frequencies of 14 loci were significantly different ($p < 0.05$) between the two populations before correction for multiple testing. After Bonferroni correction, only rs671 remained statistically significant ($p < 0.0003$). This SNP is located in the ALDH2 gene. The mutant A allele is associated with increased side effects after alcohol intake. The frequency of the GG genotype (wild type) was higher in the Okinawa Japanese (78.7%) than in mainland Japanese (34.7%; Bonferroni corrected $P<0.001$). For 31 (63.3%) of the mainland Japanese and 42 (89.4%) of Okinawa Japanese, the highest population likelihood was obtained with the Japanese reference population. However, only in a few individuals, the likelihoods were significantly different from those calculated using reference data from neighboring populations. The likelihoods for mainland Japanese and Okinawa Japanese were not significantly different from each other for any of the investigated individuals. STRUCTURE and PCA analyses showed that mainland Japanese, Okinawa Japanese, and East Asians could not be differentiated with the Precision ID Ancestry Panel.

4. APPLICATION RESEARCH OF NEXT GENERATION SEQUENCING IN THE ANALYSIS OF MIXTURE

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Currently mixture DNA analysis is still a great challenge in forensic genetics. With the development of next generation sequencing (NGS) technologies, it has been widely studied for forensic use and offers new possibilities in analysis of short tandem repeats (STR) and provides new strategies for mixture interpretation. We made 32 two-male mixed DNA samples with different mix ratio and detected them using Precision ID GlobalFiler NGS STR Panel on Ion Torrent PGM™ platform. The results showed that the majority of mixture profiles could be successfully detected even at 1:19 ratio and the NGS also presented an advantage that partial alleles from minor contributor overlapped with alleles or n-1 stutter from major contributor could be distinguished by isoalleles. We attempted to analyze the genotype of mixtures with the quantitative information of allelic reads substituting for the peak height or area of alleles from CE by a mixed DNA analysis software sepDNA designed by our lab based on two mathematical models, naïve Bayesian model and the constrained single locus analysis model. Each contributor's genotype could be accurately separated to some extend. The separation accuracy were 52.15%, 75.76%, 72.40% and 65.05% respectively using Bayesian model for different mixed ratio 1:1, 1:4, 1:9 and 1:19, which was almost same as the results get from the second model. Therefore it was feasible to analyse mixtures utilizing the quantitative information of allelic reads.

5. ASSESSING THE GENEREAD INDIVIDUAL IDENTITY SNP PANEL FOR ANALYSIS OF LOW-TEMPLATE AND PCR-INHIBITORY SAMPLES

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Massive parallel sequencing (MPS) is increasingly used for human identification purposes in forensic DNA laboratories. Forensic DNA samples are by nature heterogeneous and of varying quality, both concerning DNA integrity and matrices, creating a need for assays that can handle low amounts of DNA as well as impurities. Commercial Short Tandem Repeat (STR) analysis kits for capillary electrophoresis-based (CE) separation have evolved drastically over the past years to handle low-template samples and high amounts of various PCR inhibitors. If MPS is to be used extensively in forensic laboratories, there is a need to ascertain a similar performance. In this study we have challenged the GeneRead Individual Identity SNP panel (Qiagen) that includes 140 SNP markers1 following the GeneRead DNAseq Targeted Panels V2 handbook for library preparation, applying low levels of DNA and relevant impurities. Analysis of down to 0.1 ng DNA generated SNP profiles with at least 119 SNPs that passed quality controls, after increasing the number of PCR cycles in the initial PCR from 20 to 24. The SNP assay handled extracts from five different DNA extraction methods, including Chelex with blood and saliva, without detrimental effects. Further, the assay was shown to tolerate similar amounts of inhibitor solutions from soil, cigarettes, snuff and chewing gum as modern STR kits for CE-based analysis. In conclusion, the Qiagen forensic identification SNP PCR multiplex assay enables analysis of 0.1 ng DNA following optimization of PCR cycle number, and is compatible with several types of relevant crime scene sample types and matrices.

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6. CAN THE MPS BE USEFULL IN DISCRIMINATING QATARI POPULATION?

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Massively parallel sequencing technologies are fast going mainstream to play a major role in forensic genomics, as it is an emerging technique of choice to overcome the limitations of capillary electrophoresis (CE). By using MPS and simultaneous targeting of autosomal, X and Y STRs as well as identity and phenotypic informative SNPs, sequenced from1.0ng of DNA in single experiment. 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 data will also be vital in order to make use of the intra-allelic variability observed with sequencing. We have typed a series of 150 samples using both GlobalFiler, PowerPlexFusion6C, PowerPlexY23 and the IluminaForenSeq™ DNASignature Prep Kit 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. Results were compared for 22 autosomal and 27 anosomal STRs previously analysed and subsequently sequenced using the Forenseq DNA Signature Prep kit. 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. Subsequently, a total number of 150 unrelated different sample from Qatari populations was used to develop a database set to benchmark the ability of the selected loci to predict biogeographic ancestry.

7. CHARACTERIZATION OF SEQUENCE VARIANTS IN THE SE33 LOCUS BY MASSIVELY PARALLEL SEQUENCING

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The STR locus SE33 is one of the most polymorphic loci used in forensic DNA typing, being reported with more than 150 different alleles. The sequence structure of the SE33 locus is complex because of the sequence variations in the flanking region as well as in the core repeat region. However, current capillary electrophoresis (CE) for STR genotyping and the Sanger sequencing are limited in their ability to fully characterize the sequence structures of the SE33 locus from a large number of samples. Since a recent advance in massively parallel sequencing (MPS) made it possible, we analyzed the sequence variations of the SE33 locus from more than 300 DNA samples of African Americans, European Americans, Hispanics, and Koreans. An MPS analysis was carried out using in-house PCR panel, which simultaneously amplifies 25 autosomal STRs, DYS391, Y-M175 and amelogenin, with the small amplicon sizes ranging from 77 to 258 bp. The barcoded library for the MPS run was easily prepared by

the PCR-based library preparation method and sequenced on a MiSeq System. We compared obtained genotyping results for the SE33 locus with those obtained by the CE method, and scrutinized sequence variations in the targeted core repeat region and the flanking region. Observed sequence variants and their frequencies will be listed and statistical values will be compared among populations. The highly variant sequence information of the SE33 locus obtained through MPS analysis could be useful in solving challenging casework by increasing the discrimination power of STR loci.

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8. CHARACTERIZATION OF SEQUENCE VARIATIONS OF 29 STRS IN THE CHINESE BAI POPULATION GROUP

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Next generation sequencing-based STR genotyping provides detailed information, including DNA base substitutions and repeat sequence variations, which could not be identified by conventional CE-based methodology. Here we developed a short-amplicon (50-250 bp) multiplex system of 29 STRs (TPOX, D11S4463, D13S317, vWA, D6S1017, D3S4529, D10S1435, CSF1PO, D18S853, D2S441, D20S1082, D22S1045, D1S1627, D16S539, D1GATA113, D18S51, TH01, D3S3053, D2S1776, D1S1677, D1S1656, D5S818, D8S1179, D14S1434, D9S2157, D6S1043, D6S474, FGA and D20S482) and the sex-determining locus Amelogenin. Libraries were prepared with the Illumina TruSeq DNA PCR-Free Kits and sequenced on the MiSeq machine. Sequence-based STR alleles were called using the NextGENe v.2.4.1.2 software with allele sequences from our previous publication as reference sequences [1]. 225 individuals from the Chinese Bai population group were sequenced using this method. Sequence variations and frequencies of the STR alleles were presented, and forensic parameters between sequence-based and length-based STRs were compared, providing references for research and applications on sequence-based STR genotyping and the Chinese Bai populations.

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9. COMPARISON OF MPS AND CE BASED ANALYSIS FOR THE INTERPRETATION OF MIXED STR PROFILES

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The interpretation of short tandem repeat (STR) profiles can be challenging if, for example, alleles are masked due to allele sharing among contributors and/or when they are subject to drop-out. Interpretation with regards to estimating the number of contributors and mixture deconvolution can be improved by increasing the number of STRs and/or loci with a higher discriminatory power. Both capillary electrophoresis (CE, 6-dye) and massively parallel sequencing (MPS) provide a platform for analysing relatively large numbers of autosomal STRs. In addition, MPS enables distinguishing between sequence variants, resulting in enlarged discriminatory power. In this study, we compared the performance of both methods for the interpretation of mixed DNA profiles. To that aim, six datasets were created, each comprising 20 two- to five-person mixtures varying for the mixture proportion, level of drop-out and allele sharing. Each DNA mixture was amplified in triplicate for both the PowerPlex® Fusion 6C STR kit in combination with CE and for the MPS PowerSeq™ System, resulting in a total of 720 mixed profiles. Profiles were analysed and results for both methods were compared. Results show the ability to correctly determine the number of contributors when using the CE-generated or MPS-generated STR profiles. Moreover, deconvolution and assessing the weight of evidence of both profile types will be discussed.
This study provides a framework for the interpretation of complex mixed DNA profiles obtained using the PowerPlex® Fusion 6C STR kit in combination with CE and using the MPS PowerSeq™ System.

10. COMPARISON OF STANDARD CAPILLARY ELECTROPHORESIS BASED GENOTYPING METHOD AND FORENSEQ DNA SIGNATURE PREP KIT (ILLUMINA) ON A SET OF CHALLENGING SAMPLES

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Massively parallel sequencing (MPS) allows multiplexing of large numbers of genetic markers enabling the combination of STRs and SNPs in the same analysis. This capability is relevant especially for challenging samples where the amount of available DNA limited and can be highly degraded. For this reason, we investigated the performances of the Illumina® ForenSeq DNA Signature Prep Kit (Illumina) on a set of samples that comprised DNA extracts from FFPE samples, old bone samples (Neolithic, 7th century, 9th century, 15th century, 18th century), touch samples (mixed profile) and standard mouth swab reference samples. Primer Set B of the ForenSeq™ kit, used for this study, amplifies 27 autosomal STRs, 7 X-STRs, 24 Y-STRs, 94 identity-informative SNPs, 56 ancestry-informative SNPs, and 22 phenotypic-informative SNPs.
DNA extracted from the samples have been run both with a standard capillary electrophoresis (CE) based genotyping method and with the ForenSeq DNA Signature Prep kit. The results showed that more data can be obtained by MPS compared with CE with the same amount of input DNA and that use of MPS is influenced by the degree of damage of the template and amount of DNA available.

11. CONCORDANCE STUDY USING THE ILLUMINA MISEQ FGX NEXT GENERATION SEQUENCING SYSTEM

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Next Generation Sequencing (NGS) is a giant leap for forensic DNA analysis. In addition to a vastly increased number of identity markers, biogeographical and phenotypic genetic information can be determined. However, NGS needs to be thoroughly tested before it can be successfully applied in forensic DNA analysis. This includes validation of the technology to assess its accuracy, sensitivity and reproducibility. In addition, the concordance to established methods needs to be investigated. Lastly, application of NGS data in forensic reporting requires knowledge of allele frequencies for “classical” as well as new markers.
We used the Illumina MiSeq FGx system and DNA Primer Mix A to conduct a population study aimed at the assessment of concordance as well as the determination of allele frequencies. Based on a previous study comprising 333 unrelated persons, samples were analysed by NGS.
Using the recommended input amount of DNA (1ng), the NGS assay was very robust and sequences were obtained from almost all samples.
We observed very good concordance with the PowerPlex ESI 17 and AmpFLSTR NGM Select kits. Only a few discordances were observed for D1S1656, FGA, vWA, D12S391, D19S433, and D22S1045. The majority of these discordances could be explained by default analysis settings.
Overall, the Illumina MiSeq FGx system seems well suited for application in forensic DNA analysis, and provides its users with extensive data compared to conventional CE applications.

12. CONSIDERING A PERFORMANCE TEST OF FORENSIC GENOMICS SYSTEM ON MASSIVELY PARALLEL SEQUENCING TECHNOLOGY

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Massively parallel sequencing (MPS) technology has been recently introduced in forensic field. MiSeq FGxTM forensic genomics system (Illumina, Inc., San Diego) is the designed work flow for forensic DNA work. The system comprised of DNA library preparation kit, MPS instrument and data analysis software. In this study, 30 DNA samples of Thai population were sequenced using MiSeq FGxTM forensic genomics system. Twenty-seven loci autosomal STRs and 94 identity informative SNPs were used to analyzed. The data quality was observed according to these parameters; depth of coverage (DoC), allele coverage ratios (ACRs) and sequence coverage ratios (SCRs). For STR markers, the average DoC ranges were 523 – 11,534 reads per locus, average ARCs ranges were 0.81 – 0.95 (excluded D22S1045 with average ARCs of 0.28) and average SCRs ranges were 0.83 – 0.99. For SNP markers, the average DoC ranges were 46 – 4,012 reads per locus, average ARCs ranges were 0.54 – 0.97 (excluded rs6955448 and rs338882 with average ARCs of 0.40 and 0.49 respectively). Some of SNP markers such as rs6955448, rs338882 and rs4530059 showed high number of imbalanced individual’s sample. Fourteenth of 30 samples showed low coverage threshold at rs1736442. Intra-STR allele sequence variants were identified in 10 loci and were found in 12 of 30 samples. Finally, the STRs profiling from MiSeq FGxTM forensic genomics system revealed a concordant result with capillary electrophoresis (CE) data (using Investigator® ID plex Plus Kit - QIAGEN and PowerPlex® 16 System - Promega) in Thai population.

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13. DESIGN AND APPLICATION OF A HIGHLY VARIABLE MULTI (3 OR MORE) ALLELIC SNP SET FOR HUMAN IDENITIFCATION

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Technological advances in massively parallel sequencing have facilitated the simultaneous analysis of large numbers of genetic markers in a forensic-type scenario, i.e. where the DNA may be of limited quantity and compromised quality. Conventional single nucleotide polymorphism (SNP) analysis suffers to some degree from the di-allelic nature of most SNPs which suppresses their effectiveness when used in complex relationship/identification cases. To overcome this limitation, a set of 'multi'-allelic markers have been selected following assessment of the literature and online databases, and an assay designed incorporating 115 tri-allelic SNPs, 19 tetra-allelic SNPs and 35 microhaplotypes. This assay was applied to a set of half-sibling and cousin cases, and we present here the results from this analysis which powerfully demonstrate the potential of this marker combination when used to clarify familial relationships.

14. DETECTING DISTINCTIVE FUNGAL COMMUNITY ASSOCIATED WITH GRAVE SOIL AND RAT CADAVERS USING NGS PROFILING

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Fungal communities can be useful sources of forensic sciences because they serve important roles in decomposition in soil ecosystems [1-3]. However, difficulties in isolation and identification of fungal species are a major drawback of forensic mycology [4]. Recent advance in sequencing technology have helped overcome these problems by metagenomics using next generation sequencing (NGS) [5-6]. In this study, major fungal species associated with decaying cadavers in soil were identified using 454 pyrosequencing. We buried rats, which are model vertebrate species, in the soil and compared fungal communities between decaying samples (cadaver and grave soil) and control soil samples during a 6-week period. Pyrosequencing revealed a relatively high number of fungal OTUs in the decaying samples. Fungal diversity in cadavers was significantly lower than in grave and control soil samples. In addition, fungal communities of decaying samples were significantly different from control soil samples.

In major communities, 25 OTUs were associated with decaying samples. Moreover, some of the 25 major OTUs had a distinctive signature during temporal changes. In conclusion, we identified major fungal species associated with decaying cadavers in soil, which suggests a potential use for NGS profiling in forensic sciences such as identifying postmortem intervals.

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15. DEVELOPMENT OF 88 MULTIPLEX STR GENOTYPING SYSTEM USING MPS

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The development of massively parallel sequencing (MPS) has increased greatly in DNA sequencing. MPS allows examiners to generate data that span the human genome in a single, targeted assay. Moreover, the advantage of MPS brings to analysis of even the smallest, hard compromised, and mixed samples. The 88 multiplex STR genotyping system we demonstrated that 43 autosomal STRs loci, including the 20 expanded CODIS (Combined DNA Index System) loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433 and D22S1045) that FBI Laboratory announced in 2015, as well as 18 X-STRs loci and 27 Y-STRs loci for individual identification. The loci which except from FBI CODIS, are bring forward to elevate the accuracy of human identification. Primers generate amplification products between 115 and 290 base pair in length. The validation testing used Human Random Control DNA Panels. Data was generated both on Ion Torrent Personal Genome Machine (PGM) System and illumina MiSeq System. The small DNA amplicons with high coverage sequencing were used, the achieved correct individual identification demonstrates the suitability of this approach for analyzing degraded materials in forensic applications. As a whole, the 88 Multiplex STR Genotyping System is a well-performed, reliable and high informative MPS-STR assay. It has capabilities for individual identification and kinship analyses in forensic science in the future.

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16. DEVELOPMENT OF A NGS STR PROTOTYPE

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Next Generation Sequencing (NGS) is increasingly used as a supplementary tool to capillary electrophoresis based methods for genetic fingerprinting. The use of NGS can help to increase the discriminatory power of existing marker sets and to resolve mixtures.
We developed a prototype panel that includes all loci of the Expanded CODIS set of markers and D6S1043. The workflow employs the QIAseq 1-Step Amplicon Library Kit for one tube preparation of libraries in about 30 min. The assay is designed for sequencing on the Illumina miSeq instrument.
We will present preliminary evaluation data on sensitivity and mixtures.

17. DEVELOPMENT OF THE PRECISION ID GLOBALFILER™ NGS STR PANEL

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The Precision ID GlobalFiler™ NGS STR Panel targets 31 autosomal STRs and 4 sex-determining markers. The Precision ID DL8 Kit for Library Preparation on Ion Chef™, the Ion S5™ Precision ID Chef & Sequencing Kit for Template Preparation and Ion S5™ Sequencing Systems for sequencing were developed to sequence STRs. In concert, the new Converge 2.0 Software provides an optimized pipeline for STR Panel sequence analysis and reporting.
A study utilizing 34 known samples were sequenced with the Precision ID system workflow and concordance with previously generated CE genotyping assessed. In addition, the 32 of the samples were used to create 1:10 and 1:20 mock mixture sample sets of both genders (Male:Female, Male:Male, and Female:Female). The mixture samples were a stress test for both the STR bioinformatic algorithms and detection of a low level male contributor(s). High levels of concordance between the CE genotype and MPS genotype were seen, and resulted in accurate results displayed in simplified STR and ISFG long sequence nomenclature. Stutter ratios in the MPS system are elevated in comparison to those in a CE fragment length based system due to the multiple rounds of PCR performed. However, even with higher stutter, the more complex and compound STRs allow for determining between stutter and minor contributor when the repeat motif of the minor contributor differs significantly from the stutter allele of the major contributor.
For Forensic or Paternity Use Only.

18. ESTIMATING THE HETEROZYGOTE BALANCE OF MICROHAPLOTYPE MARKER WITH MASSIVE PARELLEL SEQUENCING

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At present, amplifying short tandem repeat (STR) by polymerase chain reaction (PCR) and subsequently genotyping the markers by capillary electrophoresis have dominated the DNA analysis in crime laboratories. Peak height which is relevant to the amount of starting DNA template is used frequently in both the interpretation of mixture DNA samples and low template DNA samples. However, many factors may affect the results (e.g., the heterozygote balance). When the smaller peak is less than 70% of the height of the larger peak at a locus, it is taken to be an indication that there is more than one contributor of template DNA. As for STR, alleles with a longer sequences are thought to present relatively smaller peak height both because they stutter more but also because they amplify less, which may destroy the heterozygote balance. Microhaplotype, which is genotyped by massively parallel sequencing (MPS), is utilized for their sequence polymorphism, and may perform better in heterozygote balance. To confirm the point above, 30 microhaplotype loci were enrolled in this study. Excluding the loci which showed poor polymorphism or much chaos in sequences, we have evaluated 12 loci for their heterozygote balance using average allele coverage ratio (ACR). ACRs were calculated by dividing the lower coverage allele by the higher coverage allele at a locus. Ten of 12 microhaplotype makers had an average ACR of 0.81-0.92. The remaining two loci had an average ACR of 0.68 (± 0.19) and 0.74 (± 0.08) respectively.

19. EVALUATION OF PRECISION ID IDENTITY PANEL ON GENOTYPING FOR TUMOR TISSUES USING ION PGM

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Short tandem repeats (STRs) are not applicable for forensic cases involving tumor tissues due to the high mutation rate.In this study, we evaluate the stability and mutation rate of single nucleotide polymorphisms (SNPs) markers in tumor tissues, The tumor tissues and adjacent normal tissues from 150 patients with cancer of breast, esophagus or stomach were detected using Precision ID Identity Panel on Ion TorrantPGM, which includes 90 autosomal SNPs and 34 Y-SNPs. We compared the performance of the panel in tumor tissues and normal tissues, such as locus coverage balance, strand balance, heterozygote balance and background noise, and the SNPs genotype. The results showed that there was severe imbalance of heterozygote in tumor tissues, compared with normal tissues. We found only one mutation type, loss of heterozygote that was observed at 23 loci in 14 tumor samples under the default threshold of HID_SNP_Genotyper plugin v4.3 indicating that about 90% of tumor samples showed consistent genotyping results with normal tissues. The mutation rate of SNPs was significantly lower than that of STRs obtained from our previous study.Therefore, SNPs would be a better choice for individual identification of tumor tissues than STRs. Increasing sequencing depth would be benefit to get more consistent results If there are only several loci showed different typing with compared samples.

20. EVALUATION OF THE EARLY ACCESS AMPLISEQ™ MITOCHONDRIAL PANEL, UTILISING A MASSIVELY

PARALLEL SEQUENCING (MPS) TECHNOLOGY

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The Early Access AmpliSeq™ Mitochondrial Panel (Life Technologies™, CA, USA) is a forensic kit that ultilises Massively Parallel Sequencing (MPS) technology to resolve whole mitochondrial genomes for matters of phylogenetic and kinship inference. However, limited information is available to assess the performance of the kit on degraded DNA - a common factor in forensic samples. The present study examines panel performance by evaluating sequences of samples that are degraded to conditions which mimic those found in forensic investigations. Five biological samples were subjected to thermal degradation at five time points each (125°C for 0, 30, 60, 120 and 240 minutes; n=25), with DNA quality assessed via gel electrophoresis and genomic DNA real-time assays. Sequencing was performed on the Ion Torrent PGM™ platform, and target sequences were obtained for all samples on an average coverage ranging between 207.11X and 906.76X. Sequence coverage was consistent across treatments (P=0.232) and well balanced between forward and reverse strands (P=0.472). Heteroplasmy was detected in five samples of which were from the same biological replicate. Of amplicons typed, 157 amplicons (97%) displayed relatively high coverages (452 ± 333X), while reads less than 100X were noticeable in 5 amplicons only. Using a coverage threshold of 10 reads per SNP, complete sequences were recovered in all samples at all treatments, and allowed kinship and haplogroup relations to be resolved. Overall, the results of our study demonstrate the efficacy of a novel MPS panel that is able to recover mitochondrial sequences from degraded DNA samples.

21. EXPLORING OF MICRORNA MARKERS FOR SEMEN STAINS USING MASSIVELY PARALLEL SEQUENCING

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MicroRNA profiling is attracting the interest of the forensic science community, for the extremely small size and differential expression pattern enable miRNAs as a promising biomarker for body fluid identification. However, the number of body fluid specific microRNAs is currently limited partly due to a limited number of microRNAs available on the microarray-based techniques. Massively parallel sequencing (MPS) has the capability of screening microRNA biomarkers for forensically relevant body fluids at the genome-wide level. Herein, we employed the Ion Personal Genome Machine System (Thermo Fisher Scientific) to characterize the distribution and expression of 2588 human mature microRNAs (miRBase V21) in forensic semen stains. An average of 2,033,000 sequence reads was generated in ten semen samples using the microRNA workflow solution developed by our previous study. Based on further analysis of miRDong V2, an improved version of miRDong, 9 microRNAs were identified as potentially semen-specific biomarkers. This study characterizes microRNA expression pattern in semen stains with an improved plugin, and propose 9 microRNAs for forensic semen samples.

22. FORENSIC OXFORD NANOPORE SEQUENCING

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One of the latest developments in next generation sequencing is Oxford Nanopore Technologies' (ONT) MinION nanopore sequencer. This pocket-sized sequencer holds some promising features that could be useful in the field of forensic genetics. In a pilot study we investigated the applicability of this novel system to perform forensic genotyping using both a 52 SNP-plex assay (SNPforID consortium) [1] and a 14-plex STR assay (based on the AmpFLSTR® Profiler Plus). Using primers originally designed for multiplex PCR and CE, we developed a method suited for nanopore sequencing. The procedure includes a barcoding step, and allows to sequence several samples in a single sequencing run. Our results show that forensic SNPs can be robustly genotyped when loci containing homopolymers in the sequences flanking the SNP are avoided. Loci with homopolymeric sequences next to the forensic SNP can lead to wrong or inconclusive results. These loci were already reported as problematic in studies using other sequencing technologies that suffer from inaccurate sequencing of homopolymers [2]. Analysis of sequencing data from the STR loci proved to be more challenging. Two analysis approaches were evaluated to extract a profile. In one approach, the sequence length was used to determine the length of the STRs. In another approach the sequences were mapped to a reference database containing the sequences of most reported forensic STR alleles. While some evidential value could be derived from the STR data, STR profiling after nanopore sequencing is currently not feasible.

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23. FORENSIC VALIDATION OF THE PRECISION ID MTDNA WHOLE GENOME PANEL USING THE ION CHEF AND THE ION S5 SYSTEM

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Mitochondrial DNA (mtDNA) is a valuable marker in forensic genetics especially with challenging samples that fail to yield results from conventional nuclear DNA markers. Massively Parallel Sequencing (MPS) is particularly helpful in this context as it provides the possibility to sequence the full mitogenome from even minute amounts of DNA bringing beneficial information for human identification and phylogenetic analysis. In this study, we evaluated the performance of the Precision ID mtDNA Whole Genome Panel in forensic and population samples. The mitogenome of all samples was sequenced using two separate amplification reactions with overlapping primer pools to cover the entire genome. The Precision ID mtDNA Whole Genome Panel contains PCR primers for 162 amplicons with an average amplicon size of 163 bp, ranging from 125 bp to 175 bp in length. Library preparation was performed using manual or automated techniques and the sequencing chips were prepared on the Ion

Chef and run on the Ion S5 System. An earlier version of the kit was tested on DNA extracts from historical bones and tooth samples to evaluate the performance on specimens containing low amounts of degraded DNA. Additionally, a total of 256 mitogenomes from various worldwide populations was sequenced to investigate primer specificity and sequencing efficiency of samples with diverse phylogenetic background.

24. GENETIC ANALYSIS OF 15TH–17TH CENTURY MUMMIES FROM GREENLAND

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Querrortuut, Pissisarfik, and Qilakitsoq are some of the best preserved Thule Culture Inuit burial sites in Greenland. The environmental conditions in these sites have allowed the natural mummification of the remains placed there and have made it possible to describe and examine several aspects of the Inuit culture and living conditions. Nevertheless, several questions are still unanswered regarding familial relationships between mummies within each site, the peopling of Greenland, and the influence of gene flow from Scandinavians. Establishing genotype frequencies for Greenlanders prior to European admixture will have forensic applications in determining proper admixture proportions and higher confidence in ancestry estimations.
Massively parallel shotgun sequencing of mummies from the three mentioned sites in Greenland allowed the recovery of complete mitochondrial genomes and typing of the same 165 SNPs already characterized for current Greenlanders (1), as well as the typical forensic identity SNPs. Mitochondrial haplotypes indicate that all of the mummies typed in this study belonged to haplogroups consistent with Inuit maternal ancestry and supporting the recovery of endogenous DNA from the mummies. Comparison of the genotype frequencies between current and historical populations will bring insight into how European colonization has affected the population genetics of Greenlanders.

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25. GENETIC IMPLICATIONS IN SUICIDAL BEHAVIOR

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Suicidal behavior is a leading cause of injury and death worldwide.
Among years, some genetic correlations have been hypothesized with a wide range of genes. A pool of 20 genes that appeared as the most significantly correlated with suicidal behavior has been selected

and an NGS panel has been developed.
The panel was applied on autoptic samples (blood or tissue material) collected among more than 20 years from suicidal patients.
The aim of the study was to verify a possible correlation between the genetic arrangement and the suicidal behavior in this selected population.
NGS data were analyzed with an internal pipeline and compared with reference genomes.

26. GENOTYPING MICROHAPLOTYPE MAEKERS THROUGH MASSIVELY PARALLEL SEQUENCING

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Short tandem repeats (STR) and single nucleotide polymorphism (SNP) are still in mainstream in forensic genetics. STR is most widely used in individual identification and Kinship analyzing, and SNP is the initial selection for inferring ancestral information. However, high mutation rate of STR and poor polymorphism of SNP limited the markers' power in practice. Microhaplotype is a new type of forensic genetic marker that encompasses more than 2 SNPs and can be sequenced and genotyped through massively parallel sequencing (MPS) platform. According to the articles published, microhaplotype can provide useful information on individual identification and Kinship analyzing. Besides, rapidly and quickly developed methodology MPS has been considered for routine use in forensics. We aimed to explore if the high throughput, high sensitivity and single-chain sequencing method of MPS could apply to disentangle mixture. We have screened out 50 candidate microhaplotype loci in a range of length from 150 to 280bp as following steps. Data was downloaded from the 1000 genome project after dealt with *VCF to PED Converter*, and haplotypes and their relevant frequencies were derived by *HAPLOVIEW*. After evaluating the amplification efficiency of 50 loci, 38 loci were enrolled in the present study. 21 mixtures that comprised 2 DNA samples (1:1, 1:9, and 1:99) were conducted in this study. Library was prepared through Wafergen platform and sequencing was conducted on Illumina® Hi-Seq platform. We tried to explore the method of disentangling mixture by analyzing the depth of coverage of alleles, and analysis is still in progress.

27. HETEROPLASMIC PATTERNS OF MITOCHONDRIAL DNA IN MIXED SAMPLES ON MASSIVELY PARALLEL SEQUENCING

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Mitochondrial heteroplasmy refers to co-existence of different polymorphisms in an individual or a cell, the pattern of which is found not only in normal inheritance, but also in pathologic status or mixed samples. Understanding of the nature of heteroplasmy will be helpful in solving the problems in forensic

mixture analysis, stem cell study or cell therapy. Massively parallel sequencing of several platforms is thought to be promising for interpretation of heteroplasmy. Here we focused on point heteroplasmic pattern of artificially mixed samples, using Ion Torrent system.
Five samples with inherent heteroplasmies of various minor base frequencies (MBFs) were selected. Then, two pairs were chosen to make mixed positions as many as possible. Each pair was differently mixed for expected MAFs of 50%, 20%, 10%, 2.5% and 1%. Two overlapping amplicons covering whole mitochondrial DNA were pooled in equal amounts for each sample. They were loaded on Ion 316 chips for sequencing and analyzed.
Average coverage was 1289X ± 713.6X (range 89X-3769X). MBFs of the inherent heteroplasmies were close to previous results, with differences under 10%. In the case of mixed positions, the overall calling rates were 100%, 87%, 58%, 33%, and 4%, respectively for the above expected MBFs, while average observed MBFs were 38.15%, 14.87%, 7.41%, 2.51%, and 2.66%.
In this study, we could obtain the results comparable with previous studies using other systems, especially including data of low level heteroplasmy. The expected and observed MBFs were directly proportional to each other generally, which suggests the possibility of future application.

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28. IMPLEMENTATION OF A FULLY AUTOMATED WORKFLOW USING ILLUMINA FORENSEQ™ SYSTEM IN A HIGH VOLUME CASEWORK LABORATORY

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Massively Parallel Sequencing (MPS) has the potential to be a promising technology for recovering genetic information from challenging DNA samples. The Illumina ForenSeq™ system is the first commercial kit applying MPS technologies to forensic genomics by targeting autosomal STRs, Y-STRs, X-STRs and a large SNP panel for human identification in a single reaction. To implement this technology in our high volume casework laboratory (238 156 casework samples analyzed in 2016), we developed, in collaboration with Hamilton Robotics, a fully automated workflow handling all of ForenSeq™ DNA Signature Prep Kit library preparation steps. Automated workflow offers high capacity (up to 96 samples) with minimal hands-on time. Validation studies, using hundreds of reference and casework samples, confirmed the robustness of this approach with regards to concordance, sensitivity, repeatability and reproducibility. The workflow is designed to enable the combined analysis of rich and poor DNA traces within the same run by adjusting each library pooling volume depending on the DNA sample concentration to ensure sufficient coverage for all samples. Casework samples analyzed with the ForenSeq Universal Analysis Software and in-house validation parameters showed that MPS technologies outperform conventional analyses for several types of samples. The workflow revealed itself to be an excellent approach to reanalyze challenging DNA samples, including mixture deconvolution, with regards to STR allelic sequence variations, and highly degraded samples. Several casework examples will be shown to highlight the synergy of MPS analyses with CE-based technologies in a high volume production laboratory.

29. INITIAL ASSESSMENT OF THE PRECISION ID GLOBALFILER MIXTURE ID PANEL ON THE ION TORRENT S5 XL DNA SEQUENCER AND CONVERGE V2.0 SOFTWARE

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The U.S. National Institute of Standards and Technology (NIST) has recently participated in an assessment of the Precision ID Globalfiler early access Mixture ID Panel (v1.0), which consists of primers for amplification of over 100 forensically relevant loci in the human genome. Markers included in the panel can be divided into four classes: short tandem repeats (STR) (29 autosomal STRs and 1 Y STR), single nucleotide polymorphisms (SNPs) (42 autosomal [1, 2] and 2 Y chromosome), insertion/deletion polymorphisms (Indels) (Amelogenin and Y Indel rs2032678), and microhaplotype blocks (MH) (36 clusters of 2 to 4 SNPs [3]). Several challenging sample types were sequenced and analyzed, including: artificially degraded DNA, multiple-contributor mixtures, mixtures of related individuals, and low DNA input samples. Performance characteristics (e.g. allele coverage ratio, interlocus balance, stutter, drop in/out, benefits and challenges of sequencing versus fragment size analysis) of the Mixture ID Panel relative to state-of-the-art capillary electrophoresis methods will be presented. Additionally, capabilities of the accompanying Converge v2.0 (beta release) analysis software will be discussed.

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30. INVESTIGATING THE EFFECTS OF DIFFERENT LIBRARY PREPARATION PROTOCOLS ON STR SEQUENCING

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Next Generation Sequencing (NGS) is transforming the landscape of the Short Tandem Repeat (STR) genotyping. NGS determines the length as well as the sequence of each allele, identifies polymorphisms in the repeats or adjacent DNA regions, allows for a greater degree of multiplexing, and generates Gigabases of reads in a single run [1-3].
An additional step introduced into the DNA typing process with NGS methods is library preparation. The PCR amplicons of the targeted loci are further purified and modified with adapters and sample specific indices prior to sequencing.

The influence of different library preparation protocols and reagents on the consistency of allele calling, depth of coverage, and allele coverage ratio was examined. In this study, the general workflow consisted of amplifying STR loci by PCR using the Prototype PowerSeq Auto System; amplicon purification; library construction and cleanup; cluster generation on the MiSeq platform; and post-sequencing data processing.
Two commercially library kits were used to perform library construction: Illumina TruSeq DNA HT Sample Prep and Kapa Hyper Prep. The selection of various methods for amplicon purification and library cleanup (e.g. columns vs. different concentration of beads) were assessed. The goal was to optimize library construction protocols to reduce bias in allele signal and allow for fast and efficient library preparation. These optimizations could lead to high-quality STR data by generating maximum number of sequencing reads and ameliorating the likelihood of alleles/loci from being poorly represented especially from mixed forensic samples and those with low amount of DNA material.

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31. INVESTIGATION OF LENGTH HETEROPLASMY IN MITOCHONDRIAL DNA CONTROL REGION BY MASSIVELY PARALLEL SEQUENCING

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Accurate sequencing of the control region of the mitochondrial genome is notoriously difficult due to the presence of polycytosine bases, termed C-tracts. The precise number of bases that constitute a C-tract and the bases beyond the poly cytosines may not be accurately defined when analyzing Sanger sequencing data separated by capillary electrophoresis. Massively parallel sequencing has the potential to resolve such poor definition and provides the opportunity to discover variants due to length heteroplasmy. In this study, the control region of mitochondrial genomes from 20 samples was sequenced using both standard Sanger methods with separation by capillary electrophoresis and also using massively parallel DNA sequencing technology. After comparison of the two sets of generated sequence, with the exception of the C-tracts where length heteroplasmy was observed, all sequences were concordant. Sequences of three segments 16184-16193, 303-315 and 568-573 with C-tracts in HVI, II and III can be clearly defined from the massively parallel sequencing data using the program SEQ Mapper. Multiple sequence variants were observed in the length of C-tracts longer than 7 bases. Our report illustrates the accurate designation of all the length variants leading to heteroplasmy in the control region of the mitochondrial genome that can be determined by SEQ Mapper based on data generated by massively parallel DNA sequencing.

32. LIMS CONFIGURATION TO FIT NEW MASSIVELY PARALLEL SEQUENCING WORKFLOWS IN FORENSIC

GENETICS

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Laboratory Information Management Systems (LIMS) are valuable tools in the forensic genetics laboratory, streamlining sample and analysis traceability, batching of multiple samples, integration and data transfer with genetic analyzers, monitoring of test results, DNA data exchange with National DNA databases, and flexible reporting. This work, developed in the framework of the DNASEQEX project, presents the configuration of a commercial LIMS system (*LabWare Inc., DE*) to fit the automated workflow provided by the *Ion Chef* / *Ion Torrent S5XL* platforms and the *Torrent Server Suite / Converge* software packages (*Thermofisher Inc., CA*) for the massively parallel sequence analysis of STR markers in the forensic lab. LIMS configurations included (1) the setup of analysis and batch templates for DNA library preparation, and DNA library quantification (2) the creation of composite samples for MPS-STR library tracking, (3) the development of files to electronically export MPS sample setups and analysis to the *Torrent Server* Software, (4) the configuration of scripts for the electronic transfer into the LIMS system of STR allele and sequence data files generated by the *Converge* software, and (5) the development of DNA data export files to national DNA databases. The developed LIMS configuration provides the laboratory with the tools required for setting up automated MPS workflows, helping to efficiently and accurately acquire and manage MPS data in the forensic genetics laboratory.

Poster Abstracts

33. MASSIVE PARALEL SEQUENCING AS A TOOL FOR MOLECULAR CHARACTERIZATION OF PORTO ALEGRE, RS, BRAZIL´S FOUNDING POPULATION

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Anthropological and archeological studies regarding ancient human remains recovered from Porto Alegre´s first original graveyard (dating back to city´s foundation in 1772) have been found insufficient for a comprehensive understanding of the town´s founding population. Molecular tools were used to answer remaining issues, as gender composition, ethnicity and admixture levels in this original population, historically considered as comprised mostly by Portuguese Azores´ settlers and their slaves (African and Native americans). Massive Paralel Sequencing was used in an attempt to further characterize this population´s molecular constitution. DNA from 14 individuals was extracted from teeth (each one at least 170 years old) manually using DNA Iq (Promega) or in the automated platform Automate Express (ThermoFisher) with the Prepfiler BTA DNA (ThermoFisher) kit. Identification and ancestry markers (Autossomal and Y chromosome SNPs) were analyzed with the Ion-PGM system, using commercially available kits (ThermoFisher). Also, mitochondrial DNA was fully sequenced for the same samples using commercial kits (ThermoFisher). No results were obtained in the SNPs analysis, due to high degradation levels (assessed by Real-Time PCR) in the retrieved DNA. Full MtDNA sequences were obtained for 9 of the 14 individuals, even with DNA quantities below 0.5 ng per reaction. Some samples were sequenced twice, in a different facility, resulting in the same consensus sequences. MtDNA haplotypes were classified as African (n=7) and Native American (n=2) origin. A medium coverage for the samples (over 350x) was achieved in a simultaneous 48 samples per chip

run. Further efforts are being directed to improve the extraction method.

34. MASSIVELY PARALLEL SEQUENCING OF FORENSIC SAMPLES USING PRECISION ID MTDNA WHOLE GENOME PANEL ON THE ION S5™ SYSTEM

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Mitochondrial DNA (mtDNA) has unique features such as absence of recombination and multiple copies per cell, enabling the analysis of compromised samples where nuclear DNA is insufficient in quality and/or quantity. In this study, mtDNA analysis of a variety of samples was performed by Massive Parallel Sequencing (MPS) on the Ion S5™ System. Reference and casework samples were extracted with Chelex® 100 and PrepFiler®, respectively. Samples were quantified with Quantifiler® Trio and the optimum amount of mtDNA was estimated by using as reference the input of approximately 0.1 ng of nuclear DNA. Library preparation was performed using Precision ID mtDNA Whole Genome Panel and Ion AmpliSeq™ Library Kit 2.0, followed by quantification of the unamplified library with Ion Library TaqMan™ Quantitation Kit, according to the user's guide. All barcoded libraries were diluted in equimolar volumes of 7.5 pM to ensure equal contribution in the sequencing run. The final pool was subjected to emulsion PCR on Ion OneTouch™ 2 using Ion 520™ & 530™ Kit-OT2, followed by template-positive Ion Sphere Particles enrichment on Ion OneTouch™ ES. Finally, loaded chips were sequenced on the Ion S5™, according to the manufacturer's recommendations. Raw data were analyzed with the Ion Torrent Suite™ Server v.5.5.5 using the Torrent Variant Caller plugin v.5.2.13.9. Binary alignment map (BAM) and binary alignment index (BAI) files were visualized using the Integrative Genomic Viewer v.2.3. The results showed that mtDNA haplotypes obtained with MPS were consistent with those previously obtained with traditional Sanger sequencing, regardless of the extraction method used.

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35. MASSIVELY PARALLEL SEQUENCING OF FORENSIC STRS USING THE ION CHEF™ AND THE ION S5™ XL SYSTEM

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STR data yielded with several NGS platforms has been published, but forensic application trials on the Ion S5™ XL system has not been reported. In this work, we report our preliminary data on the Ion Chef™ and the Ion S5™ XL systems using an early access panel multiplexing 24 STR markers and the sex-determining locus Amelogenin. Sensitivity experiments showed that sequencing data with 62 pg of genomic DNA was still acceptable. Mixture studies demonstrated that alleles from the minor contributor could be correctly assigned at 1:9 and 9:1 ratios. In the parallel experiments using simulated degradation samples, NGS proved to be advantageous over capillary electrophoresis (CE) in genotyping the severely degraded samples (DI=8.947). Notably, NGS successfully reported 12 full genotype results in 13 challenging casework samples, compared with 5 full results on the CE platform. Interestingly, discordant STR genotypes were observed when using different softwares (Next*GENe*, STRait Razor and Ion Torrent Suite) in analyzing the same FASTQ data suggesting that computer programing and bioinformatic parameters could impact NGS-based STR results substantially.

36. MASSIVELY PARALLEL SEQUENCING OF HUMAN BONE SAMPLES USING THE MISEQ® FGX™ AND HID-ION™ PGM SYSTEMS

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Current widespread approaches to human DNA identification are often challenged in providing enough information for reliable evaluation especially when samples contain low DNA quantity and quality such as those recovered from human bone samples. Recent advancements with Massively Parallel Sequencing (MPS) technology allows for the simultaneous analysis of different genetic marker systems i.e. Short Tandem Repeats (STR's), single nucleotide polymorphisms (SNP's), and mitochondrial DNA markers from limited DNA extracts. MPS data adds more discriminatory power to DNA testing results that are essential for cases of human identification especially from mass disasters.
In this study, we compared genotyping performance of MiSeq® FGxTM (Illumina) and HID-Ion PGMTM Systems (ThermoFisher) using femur and bone samples from recently deceased and exhumed human remains. In addition, we evaluated MPS data of DNA obtained from metatarsals (bones of the feet) which are easier to collect in mass disaster situations. Concordance of the genotype calls, percentage of allele calls, coverage reads, and balance of heterozygous alleles across the two MPS platforms will be reported. Results aim to maximize the benefit of the testing power of MPS, overcome the challenges of genotyping degraded samples and limitations in the use of capillary electrophoresis testing in resolving a wider spectrum of forensic cases where bone samples are used as the DNA source.

37. MASSIVELY PARALLEL SEQUENCING OF STRS AND MICROHAPLOTYPES FOR MIXTURES

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Microhaplotypes are multi-allelic genomic markers that contain more than one SNP residing in genetic proximity, whereby each haplotype becomes an allele in a phased genotype1. These markers provide an ideal tool for mixture analysis, with a lower baseline and better intralocus balance over STRs in a massively parallel sequencing (MPS) context and without the complexity of stutter. On the other hand, MPS exposes the additional diversity in compound and complex STR loci when repeat and flanking sequence is compared2,3,4. To investigate the sensitivity of the two marker types for mixture detection, a range of major to minor donor ratios and number of individuals was sequenced.
78 microhaplotypes that have been previously typed on 83 populations5, showing high number of alleles and ancestry informativeness were targeted for design in multiplex with 31 autosomal STRs and 4 Y-markers. DNA was extracted from samples taken from individuals of different biogeographic ancestries. Mixtures were created at ratios of 1:1, 1:3, 1:10, 1:20, and 1:50 along with mixtures containing up to 4 donors. Libraries for MPS were barcoded and sequenced on the Ion S5™. Reads were aligned to target regions of the reference human genome, and haplotypes, biogeographic ancestry, number of contributors, mixture ratios, and minor and major contributors were determined.
With the capacity to sequence many markers in parallel, MPS underscores the power of microhaplotypes and STR sequence as powerful forensic markers for handling mixture samples where CE systems are not capable of generating conclusive results.
For Forensic and Paternity Use Only

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38. MASSIVELY PARALLEL SEQUENCING OF THE MITOCHONDRIAL DNA CONTROL REGION FROM BONE SAMPLES COMPARED WITH CONVENTIONAL SEQUENCING

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Bone is one of the most typically encountered evidence types in forensic casework and missing person identification. Especially, in missing person identification cases, ancient remains investigations and mass disasters, bone plays the important role as the best biological material available for DNA typing. Unfortunately, bone taken from decomposed dead bodies cannot recover enough DNA for STR analysis. Mitochondrial DNA analysis is an alternative tool to human identification. Recently,

conventional Sanger sequencing has been a routine workflow for mtDNA analysis. The introduction of massive parallel sequencing (MPS) has promoted the mtDNA genome typing more available for routine analysis. Due to the high throughput of MPS novel genetic data improved discrimination power for identification. This study, we have evaluated the performance of mtGenome sequencing of bone samples using the Ion Torrent Personal Genome Machine (PGM) technology and compared the resulting haplotypes directly with conventional Sanger sequencing. With the focusing on the control region, the results showed the mtGenomes data yielded high concordance with the corresponding conventional Sanger sequencing haplotypes. The differences were observed the homopolymeric sequence stretches. The acquisition of mtGenome data from bone sample in this study is a preliminary development of mtGenome data from bone evidence followed by numerous further studies.

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39. MITOCHONDRIAL DNA ANALYSIS USING MASSIVELY PARALLEL SEQUENCING SYSTEMS

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Massively parallel sequencing allows laboratories access to mitochondrial DNA (mtDNA) analysis using a simpler, yet potentially high-throughput workflow. Increased mixture deconvolution and heteroplasmy resolution are achieved by deep sequencing coverage and digital read counts, compared to traditional sequencing methods. Additionally, the use of small amplicons to sequence the mitochondrial control region improves sequencing results from degraded samples. However, library preparation for massively parallel sequencing requires multiple enzymatic and purification steps spanning 3.5 hours elapsed time and is often a source of variability and sample loss.
The prototype PowerSeq™ Mito Control Region, Nested System generates 10 small amplicons (adapted from Eichmann and Parson) covering the control region of the mitochondrial genome in one multiplex. The amplicons are designed to be in a range of 140-300bp to ensure optimal results from degraded samples. The protocol consists of a single PCR step to both amplify the target amplicons and incorporate indexed sequencing adapters. This nested amplification protocol greatly reduces library preparation steps and time needed. We will demonstrate this improved workflow for the nested amplification of mitochondrial HVI and HVII control regions.

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40. MITOCHONDRIAL GENOME AND NUCLEAR SNP PROBE CAPTURE NEXT-GENERATION SEQUENCING SYSTEM FOR ANALYZING DEGRADED AND MIXED DNA SAMPLES

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Forensic biological samples can often be highly compromised (degraded, limited, or mixed). Alternative markers such as nuclear Single Nucleotide Polymorphisms (SNPs) or mitochondrial DNA (mtDNA) can be analyzed for increased genotyping success in cases where STR analysis using conventional electrophoretic methods fail. Recently, next-generation sequencing (NGS) methods have been developed which have the potential to overcome many of the limitations of conventional methods used for analyzing mtDNA and SNP markers. Recently, we have developed probe capture NGS systems for massively parallel sequencing the entire mitochondrial genome and over 450 nuclear polymorphisms for the analysis of highly degraded, limited, and mixed samples. This approach uses DNA probes to enrich targeted regions from randomly fragmented DNA libraries for clonal, massively parallel sequencing, thereby maximizing recovery of short DNA fragments characteristic of forensic samples. The clonal sequencing aspect of NGS allows for analysis of the components of a mixture separately and by counting the number of sequence reads assigned to each individual contributor of the mixture. We have successfully applied this system to sequence the entire mitochondrial genome of limited and highly degraded DNA from hair and bones as well as mixtures. A software program (mixemt) was developed which uses an expectation-maximization algorithm to resolve major and minor haplotypes in a mtDNA mixture using phylogentic information and applied to mtDNA mixture analysis. We have also demonstrated proof of concept of the nuclear SNP capture NGS system for analyzing degraded DNA including a telogen hair as well as mixed DNA samples.

41. NEXT-GENERATION SEQUENCING OF 74 Y-SNPS TO CONSTRUCT A CONCISE CONSENSUS PHYLOGENY TREE FOR CHINESE POPULATION

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The development of a concise consensus tree with high resolution for forensic genetic studies is highly recommended within the current next generation sequencing (NGS) era. In this study, a phylogeny with 74 Y-SNP markers, including two new markers with considerable polymorphism in the Han population, was constructed using the Ion Personal Genome Machine®(PGM) System, and 100 samples from the Sichuan Han population were analyzed and assigned to 18 haplogroups. Compared with the full

reference Y-Chromosome Consortium tree, this new concise consensus tree covered almost all of the Chinese Y-haplogroups and can be used to determine accurate hierarchal positions of any Chinese individual. Another notable finding from the present study was to ascertain the approximate locations of two new markers in the existing phylogeny. Also, 17 samples were observed as No-Calls in two loci (P31 and M133) in PGM system and discussed. The phylogeny in this study could be applicable for forensic purpose in Chinese population.

42. NGS-BASED STR TYPING IN PATERNITY TESTING

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As the advances of next-generation sequencing (NGS) technology, whether it is an improved solution for STR typing in paternity testing compared with traditional capillary electrophoresis (CE) is worth studying. In this study, 42 blood samples from 14 trio parentage testing cases with mutation events were analyzed with Early Access STR Kit based on Ion PGM platform. The depth of coverage (DoC), percentage of DoC, stutter ratio, stutter occurrence ratio for each locus, allele coverage ratio (ACR), and paternity index (PI) were analyzed. The results showed that NGS-based and CE-based STR typing revealed a high concordance (99.11%). The discordances were mainly attributed to the heterozygote imbalance (ACR<0.6), low DoC of alleles (<50 reads), primer differences or nomenclature strategies. Limited by the small sample size in this study, no significant improvement was observed for cases with unassigned mutations, but its potential capability to distinguish the ambiguous mutation direction, step and origin was still expected. And the different allele recognition between NGS-STR and CE-STR approaches may result in different PI calculated formulas and substitution of allele frequencies. Because of the existence of isoalleles, NGS-based STR typing has the potential capability to increase the PI value.

43. PARALLEL SEQUENCING OF X-CHROMOSOME GENETIC MARKERS

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X chromosome genetic markers are widely used in forensic genetics, for example, deficiency kinship cases, where the putative father cannot be typed. Commercial kits of X-chromosome short-tandem repeats (X-STRs) were developed and popular applied, such as Investigator X-12 kit (Qiagen, Germany). However, the use of X-chromosome single-nucleotide polymorphisms (X-SNPs) and Insertion/Deletions (X-InDels) are still limited. In this study, we sequenced 12 X-STRs included in Investigator X-12 kit, 30 X-SNPs and 18 X-Indels with massively parallel sequencing (MPS). Primers were designed with Primer Premier 5 and Oligo 3. With prepared libraries, multiple samples of the 60 X-chromosome markers are sequenced in parallel. Sequencing results of the three different genetic markers were consistent with genotyping results from capillary electrophoresis (CE), demonstrating the reliability of the custom-designed panel and the MPS technology. High coverage sequencing data were

used to determine the allele/stutter/noise ratio and the allele coverage ratio (ACR) values for the heterozygous genotypes at the 12 X-STRs. Full concordance was seen with the DNA input down to 0.5 ng. However, an increased percentage in heterozygous imbalance was observed when the input DNA amount was lower than 1 ng. The allelic sequences obtained with the MPS technology reveal true variations in the X-STR loci and identify previously unknown alleles. The whole study combining STRs, SNPs and InDels provides an effective solution for genetic markers detection on X chromosome and allows more forensically relevant information to be obtained from limited forensic material.

44. PERFORMANCE COMPARISON OF MISEQ FORENSIC GENOMICS SYSTEM AND STR-CE USING CONTROL AND MOCK IED SAMPLES

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The adoption of next-generation sequencing (NGS) for forensic purposes are on the rise. Studies have shown that NGS can replace or supplement conventional STR-CE system, but direct comparisons between them are still lacking, especially on improvised explosive device evidence. Here, we directly compared the performances of the MiSeq Forensic Genomics System (ForenSeq kit on the MiSeq FGx instrument utilizing the Universal Analysis Software with the Identifiler Plus kit on the ABI3130/3500 instrument. Dilution series (500 to 8 pg), mixture samples (1:1 to 1:100), inhibited samples (humic acid and calcium) and touch DNA on mock IED substrates were used. Profile qualities were highly correlated between the two systems. The MiSeq system was equally sensitive to the STR-CE system for single-source samples, but it was more sensitive to low-level mixtures. Alleles from the minor contributor were differentiable even at 1:50 ratio. At high concentrations of humic acid, the STR-CE system was more robust, but this trend was reversed with calcium. High quality profiles were obtained from touch DNA samples on various IED substrates, even at sub-100 pg concentrations. Due to the sheer number and variety of loci typed, higher likelihood ratios were obtained with the MiSeq profiles and more adjunct information, such as Y-STRs and identity SNPs, are available. The intra-STR sequence variants also allowed unambiguous designation of stutters and true alleles. These suggest that the MiSeq system can be used for touch DNA samples from IED substrates. Further evaluations of the MiSeq system using real IED casework samples are ongoing.

45. PERFORMANCE TEST OF PROTOTYPE POWERSEQ™ AUTO/Y/MITO SYSTEMS FOR NGS

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Capillary Electrophoresis(CE) based short tandem repeat typing is gold-standard method for forensic human Identification fields. Recently, however, CE based STR typing method has been challenged by massively parallel sequencing method. Since the massively parallel sequencing(MPS) method was developed, it has been focused on the possibility to beyond some limitations of traditional CE-based method via detection of intra-sequence variation within STRs or their flanking regions, marker expansion, provide different type of genetic marker with STR. Accordingly, various MPS based HID kits are developed, these applications provide the chemistry which has capability to amplify multiple types of markers such as STR and SNP in a single reaction, and sequencing multiple samples per one run. The prototype PowerSeqTM Auto/Y/Mito Systems which has capability to amplify 23 Autosomal STR loci, 21 Y-STR loci and Mitochondrial hyper variable region simultaneously. And also designed for Illumina TruSeq® library preparation systems, and illumina MiSeqTM sequencer. In this study, we examined this prototype systems using 16 reference type samples with 2800M control DNA. In addition, we calculate several QC parameter, and investigate sensitivity, reliability. These data shows well balanced multiplexing environment and relatively low PCR bias. Raw Fastq files, generated by the MiSeq sequencer, were analyzed by StraitRazor 2.0 and NextGene software(trial version), and the results are mostly concordant with CE data include mitochondrial genotype with 0.5ng of DNA.

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46. POWERSEQ™ 46GY SYSTEM: COMBINING AUTOSOMAL AND Y-CHROMOSOME STRS FOR FORENSIC DNA ANALYSIS USING MASSIVELY PARALLEL SEQUENCING

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Massively parallel sequencing is gaining increased interest in the forensic community. Historically, STR-based systems validated for forensics generate fluorescently-labeled amplicons that are separated by capillary electrophoresis (CE). With CE, allele calling is based on the size and fluorescent label of the amplified alleles. Massively Parallel Sequencing (MPS) based methods offer several advantages over CE methods: identification of sequence polymorphisms, more accurate quantitation of alleles, the ability to analyze more loci and the ability to make all amplicons smaller.
PowerSeq™ 46GY System includes primers and amplification master mix for sequencing autosomal short tandem repeats (STRs) and Y-chromosome STRs in a single assay on an Illumina MiSeq® System. The selected STR loci include the new CODIS core loci as well as 23 Y-STR loci that are routinely used for capillary electrophoresis-based forensic DNA analysis. The combination of these STR loci and Amelogenin makes this multiplex an effective tool for human identification using Massively Parallel Sequencing and maintains compatibility with existing databases worldwide. To ensure optimum results, quantification of the library is important. Using qPCR technology, the PowerSeq™ Quant MS System allows for determination of the concentration of next-generation sequencing libraries generated with the PowerSeq™ Systems. The PowerSeq™ Quant MS has been optimized for use with the PowerSeq™ 46GY System that is compatible with Illumina MiSeq® platforms.
Data including sensitivity and mixture data for PowerSeq™ 46GY System as well as precision data for PowerSeq™ Quant MS will be presented demonstrating that the performance of the PowerSeq™ Systems is suitable for forensic analysis.

47. PREDICTING STUTTER PROPORTIONS USING THE BLMM

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Stutters are a common and well documented artefact of the amplification of short tandem repeat (STR) regions when using a polymerase chain reaction (PCR) occurring as strands one motif shorter or longer than the allele. As the PCR amplification of STR regions is a necessary part of analysing DNA samples, understanding the mechanism and rate with which stutters are created is important. This is particularly important when the samples contain small amounts of DNA, or DNA from multiple contributors.
It has been shown that there is a linear relationship between the longest uninterrupted stretch (LUS) and the stutter proportion (a measure of the rate of stuttering). This holds if there is only a single stutter. However, with massively parallel sequencing (MPS), we see that alleles may create multiple stutters, which would have the same LUS. Thus, we need to refine the LUS, working of the same hypothesis.
The hypothesis underlying stutters is simple: The more repetitive a DNA strand is, the more likely the PCR process is to stutter. Therefore, instead of looking at just the LUS, we look at any uninterrupted stretch, called a block, and identify from which block the stutter came. We define the block length of the missing motif (BLMM), as the length of this block. We show that when switching from LUS to BLMM, the predictive accuracy increases by a factor close to 2.

48. PROBABILISTIC GENOTYPING OF SEQUENCE-DERIVED STR DATA

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Massively parallel sequencing (MPS) technologies continue to be investigated for forensic application, and have the potential to improve typing of short tandem repeat (STR) loci in multiple respects. Some of the expected advantages of MPS for STR typing include enhanced mixture detection and genotype resolution via sequence variation among alleles of the same length. However, at the same time that MPS methods for forensic DNA typing have advanced in recent years, many caseworking laboratories have implemented or are transitioning to probabilistic genotyping to improve interpretation of their autosomal STR results. Current probabilistic software programs are designed for data produced using length-based typing technologies (i.e. capillary electrophoresis), and do not accommodate sequence strings or other common shorthand notations of the sequence (e.g. a bracketed format) as the signal input. Yet to leverage the benefits of MPS for enhanced genotyping and mixture deconvolution, the sequence variation among same-length products must be utilized in some form. We will present research exploring strategies for the transformation of sequence-based autosomal STR typing results to permit their interpretation in a probabilistic framework. These include use of the longest uninterrupted stretch (LUS) length as a mechanism to represent some sequence variation that occurs within the core repeat regions.

49. PROGRESS WITH THE ALIGNMENT, ANNOTATION AND NOMENCLATURE FRAMEWORKS FOR FORENSIC STRS ANALYZED WITH MPS

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At the 26th ISFG congress in Krakow, 2015, the ISFG began the process of consultation with the forensic genetics community and establishment of simple guidelines, on the compilation of MPS-based sequence variation in the commonly used forensic STRs. From these initiatives, the ISFG DNA commission on minimal nomenclature requirements was formed. In early 2016 the commission published a set of guidelines [1] for: sequence alignment (an agreed single sequence strand aligned to the GRCh37 and GRCh38 human genome builds); variant annotation (compilation of known variants and sequence variation in the full sequence string obtained from MPS analyses); and a minimal nomenclature system (covering the whole sequence string and anticipating hitherto unrecorded variation).
Here we report updated frameworks for alignment and annotation, with emphasis on the characteristics of repeat region variation known to occur in the common forensic STRs. The current human genome reference sequence data for 37 autosomal STRs; 34 Y-STRs; and 17 X-STRs has undergone continuous adaptation towards a consistent and rigorous set of sequence descriptions. These descriptions are now available to the community as a date-stamped Excel file, placed in a dynamic FTP site which allows downloading of the latest set of STR sequence frameworks (http://strider.online/nomenclature). This allows for expanded STR sets that add new loci; changes to variant annotations in 1000 Genomes or dbSNP; and any future changes to STR repeat allele descriptions recommended by the ISFG commission.

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50. QIAGEN RAPID MASSIVELY PARALLEL SEQUENCING (MPS) WORKFLOW

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Massively parallel sequencing (MPS) of identity informative single nucleotide polymorphisms (IISNPs) offers a number of advantages over capillary electrophoresis (CE) of short tandem repeats (STRs) for forensic identity. Firstly, SNPs can be included in smaller polymerase chain reaction (PCR) amplicons, making them more sensitive than STRs for degraded DNA. Secondly, PCR multiplexes can be much larger for MPS than for CE. Thirdly, MPS is more sensitive than CE, depending on the coverage

devoted to each sample. Finally, MPS offers economies of scale for high throughput sequencing of multiple pooled samples.
The GeneRead DNAseq 140 IISNP MPS panel (QIAGEN) has been evaluated on both the MiSeq (Illumina)1 and Ion PGM (Thermo Fisher Scientific)2 MPS platforms using the GeneRead DNAseq Targeted Panels V2 library preparation workflow (QIAGEN). Here we demonstrate the implementation of a rapid and streamlined workflow for this panel consisting of direct-to-PCR addition of DNA followed by the QIAseq cfDNA Library T Kit (QIAGEN) half-hour library preparation. This revised protocol drastically reduced the time required to produce MPS genotypes from DNA swabs and FTA paper.

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51. REPRODUCIBILITY OF METHYLATED CPG TYPING WITH THE ILLUMINA MISEQ

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Epigenetics is an important research field within forensic genetics. Currently, epigenetic analyses are used for identification of body fluids and for estimation of the age of humans. Here, we evaluated some of the challenges and pitfalls of studying methylated CpG sites. We compared the methylated CpG analysis of two different methods: 1) massively parallel sequencing using the Illumina MiSeq and 2) the iPLEX assay on the MassARRAY® System. On the Illumina MiSeq, the standard variation of the fraction of methylation, $5^{me}C/(5^{me}C+C)$, was only 1% between replicates, whereas the reproducibility of the MassARRAY® was very difficult to achieve. We tested three commonly used bisulfite conversion kits: The EZ DNA Methylation-Gold™ Kit (Qiagen), the MethylEdge® Bisulfite Conversion System (Promega), and the Premium Bisulfite kit (Diagenode). We found that the Premium Bisulfite kit was superior to the other two kits and left the bisulfite treated DNA more intact as the amplification success of the downstream reactions was highest with this kit. Shorter bisulfite incubation time resulted in less DNA damage and improved the amplification success. Preliminary results showed that the incubation time could be reduced from 60 to 30 minutes without affecting the $5^{me}C/(5^{me}C+C)$ fraction in CpG sites and for cytosines that are not positioned 5' to a guanine.

52. SELECTION OF UNIVERSAL IISNPS FOR HUMAN IDENTIFICATION FROM FORENSIC PANELS OF MASSIVELY PARALLEL SEQUENCING PLATFORMS

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The capability of the massively parallel sequencing (MPS) platforms, such as MiSeq® FGxTM (Illumina) and HID Ion PGMTM (ThermoFisher), to process hundreds of SNPs in a single run has significantly increased the Power of Discrimination (PD) of Individual Identification SNPs (IISNPs) to a value even higher than the PDs of current routine STR assays. SNPs are essentially more effective than STRs when genotyping samples that contain highly degraded DNA. In addition, IISNPs also have the potential to create a database that provides match probabilities irrespective of ancestry (Fst<0.06) making them even more desirable for forensic applications. This database utilizes universal IISNP markers that are highly informative (heterozygosity >0.4) thus requiring fewer number of IISNPs to reach high enough PD [1]. In this study, we selected universal IISNPs from commercially available SNP marker panels, ForenSeqTM DNA Signature Prep Kit and HID Ion AmpliSeqTM Identity Panel. We identified 64 universal IISNPs out of 101 IISNPs with Fst<0.06 and heterozygosity>0.4. Discriminant analysis of principal components using the 64 universal SNPs showed 15 populations from 1000 Genomes Project of the SNPforID Browser clustering into one group. The remaining set of non-universal SNPs carried enough information to infer if an individual is of Asian, African, or European-American ancestry. PD of the 101 IISNPs (1-3.752 x 10-37) and 64 universal IISNPs (1-1.219 x 10-25) for the Filipino population were calculated to be higher than the PD of 20 STRs from the expanded CODIS markers (1-1.78 x 10-23) of the same population [2].

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53. SEQUENCING OF THE HIGHLY POLYMORPHIC STR LOCUS SE33

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The details of sequencing and analysis of U.S. population samples for the uniquely complex Short Tandem Repeat (STR) locus SE33 will be presented. The NIST U.S. Population Sample Set consists of 1036 unrelated individuals. There are four population groups represented: African American (n = 342), Asian (n = 97), Caucasian (n = 361), and Hispanic (n = 236). These samples have been analyzed using next generation sequencing technology targeting important STR sequences commonly used for human identification. The analysis of SE33 included in this data set required a customized bioinformatic approach to identify and process the allelic information. The locus SE33 is one of the most polymorphic markers used by the forensic community [1]. SE33 is a highly variable locus by length and sequencing has resulted in a four-fold increase in the number of observed alleles. The NIST Population Sample Set has an observed range of 6.3 to 36 tetranucleotide repeats [2]. It has 52 unique alleles by length and 264 unique alleles by sequence. Analysis of this data set shows greater then 99% concordance with length based methods when flanking sequence is considered. The different categories (classes) of repeat motifs revealed will be illustrated and further stratified by population group.

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54. SNP TYPING USING THE HID-ION AMPLISEQTM IDENTITY PANEL IN A SOUTHERN CHINESE POPULATION

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Single nucleotide polymorphisms (SNP), with lower mutation rates and smaller amplicon sizes compared with short tandem repeats (STR), is potential useful tool in forensic human identification as well as forensic DNA phenotyping. As bi-allelic markers, the per-locus discrimination power of SNP is weaker than that of STR, which could be compensated by typing larger number of loci. In this study, 90 autosomal SNPs and 34 Y-chromosomal SNPs were sequenced simultaneously using HID-Ion AmpliSeqTM Identity Panel on the Ion PGMTM platform. A total of 125 samples from a southern Chinese population were studied. Forensic parameters were calculated and haplogrouping concordance was assessed based on Y-SNP haplotypes and Y-STR haplotypes. The results showed that loci with lower coverage tend to have more frequent allelic imbalance. 0.28% of the reads was background signals. The number of reads with incorrect allele was 4.6 folds of that of nucleotide calls which differed from the SNP genotype call. The percentage of incorrect allele was associated with genetic diversity of the SNP and is lower in major allele than in minor allele. The combined discrimination power (CDP) was 1−4.81×10-34, the combined power of exclusion (CPE) was 0.99989 and 0.99999992 for duo and trio paternity testing, respectively. No significant genetic difference was observed between southern and northern Chinese populations. For haplogroup study, O3 was the predominant haplogroup and 70.3% of samples were assigned to the same haplogoup with Y-SNP and Y-STR haplotypes. In conclusion, the AmpliSeqTM identity panel was powerful for individual identification and trio paternity testing.

55. STR FLANKING REGIONS VARIATION REVEALED WITH FORENSEQ^{TM} DNA SIGNATURE PREP KIT IN POLISH POPULATION

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Examining short tandem repeat (STR) markers using massively parallel sequencing (MPS) provides a new opportunity in forensic DNA typing for increasing informativeness of these markers. Also, DNA sequence of STR flanking regions can increase discrimination power and improve mixture analysis. In

this study 450 individuals from Polish population were sequenced using Illumina MiSeq FGx sequencing platform and ForenSeqTM DNA Signature Prep Kit. Library preparation and sequencing workflow was consistent with manufacturers protocols. Obtained data were analyzed with custom bioinformatics tools. The analysis revealed presence of SNPs in the studied flanking regions. Comparative analysis with Wendt et al. showed that part of polymorphisms are located in hotspots while some are new variants. Our study confirms that there is still a hidden information available in the flanking regions of STR systems included in the ForenSeq kit that can be retrieved using custom approach and increase informativeness of human identification analysis using STR systems examined with MPS.

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56. STRENGTHS: AN ANALYTICAL TOOL FOR STR PROFILING BY REGULAR EXPRESSION AND ALIGNMENT USING HIGH-THROUGHPUT SEQUENCING DATA

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Traditionally, the identification of Short Tandem Repeat (STR) has been conducted based on the length of the PCR product using capillary electrophoresis (CE). With the advent of the massively parallel sequencing (as known as the Next-Generation Sequencing), we could now observe the exact sequences around the STR to determine not only the type of allele but the genomic polymorphisms within the region for a better identification of individuals. Here, we introduce STRengths, a tool that deconvolutes NGS reads by the primer sequence, and then detects sequence variants in the flanking and repetitive regions using pairwise sequence alignment. STRengths provides valuable information about the target STR regions such as the result of primary allele typing (based on sequence) and secondary allele typing (based on length), sequence variants in the flanking regions, and the pattern of repeats. STRengths also identifies the alteration of repeat patterns induced from the sequence variation, and calculates a proportion of noise and stutter by using read count, which may represent the quality of sequencing. STRengths showed a comparable or better sensitivity in the performance test and was faster than any other tools. STRengths is made available in two interfaces: graphical and command line. Both versions are available from https://sourceforge.net/projects/strengths/.

57. SYSTEMATIC EVALUATION OF MASSIVELY PARALLEL STR SEQUENCING IN THE DNASEQEX PROJECT

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Massively parallel (next generation) sequencing (MPS) technologies revolutionized genomic and genetic research and finally have arrived in forensic genetics. By adding new sequence information to conventional size-based STR typing MPS will increase the power of discrimination of many forensically relevant markers. Additional beneficial features include, i) an increased number of loci that can be simultaneously analyzed, ii) detailed information regarding potential DNA sequence variation among alleles of equal length, and iii) elucidation of the exact structure of micro-variant alleles. To comply with the high quality standards of forensic genetics, MPS must be rigorously validated with respect to robustness and performance to ensure reliable and reproducible results. These benefits and requirements are being addressed within the EU-funded project DNASEQEX (DNA-STR Massive Sequencing & International Information Exchange) in a collaborative way. Samples were analyzed using either the MiSeq FGx benchtop sequencer (Illumina Inc., CA) or the Ion S5 sequencing system (Thermo Fisher Scientific Inc., CA) to evaluate and validate their capability for routine applications. Library preparation, purification, normalization and pooling were manually performed for all samples amplified using the ForenSeq DNA Signature Prep Kit (Illumina, CA) according to the manufacturer's instructions. For samples analyzed using the Ion S5 sequencing system library and template preparation plus chip loading were performed using automated techniques on the Ion Chef instrument according to the manufacturer's instructions. Forensically relevant parameters such as allele calling, sensitivity, reproducibility, and concordance, were tested on forensically relevant samples including mock casework, mixtures, and a population study.

58. THE DETAILED EXAMINATION OF THE HUMAN ANCIENT MITOCHONDRIAL DNA USING THE ION PGM™ SYSTEM

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An archaeological site that has three cemeteries was found at northeastern part of Japan. A total of 45 human skeletons were excavated from the site and dated to about early 18th to mid-19th centuries based on the archaeological evidences. In the previous study1), three samples were excluded from the result because the partial mtDNA sequences of these samples were identical to those of the two laboratory personnel who were in charge of skeletal reconstruction. In this study, the whole mtDNA sequences of these three samples were amplified by the Precision ID mtDNA Whole Genome Panel kit, followed by the massively parallel sequencing using the Ion PGMTM System (Thermo Fisher Scientific). We then got the 1,684,507 reads, 11,466 depth and 97.14% uniformity on average. The three samples shared the same whole mtDNA sequences with 38 mutations as compared to the rCRS, and assigned to haplogroup D4a1c. These results suggested that the three individuals might belong to the same maternal line. The haplogroup D4 is the most dominant in Japan (more than 30 %). Therefore, matrilineal relationship between the individuals cannot be fully clarified by the conventional partial sequencing of mtDNA. Thus the whole mtDNA sequencing by using Ion PGM system is considered to be highly effective to shed light on the matrilineal relationship of the ancient human skeletons.

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59. THE MASSIVELY PARALLEL SEQUENCING OF BACTERIAL 16S RIBOSOMAL RNA GENE FROM URINE SAMPLES FOR FORENSIC APPLICATIONS

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The recent identification of microbiota in the human bladder has emphasized the importance of bacterial analysis of urine as a method for predicting urinary-tract related diseases as neurogenic bladder dysfunction and interstitial cystitis [1]. In the context of forensics small tandem repeat (STR) and microRNA (miRNA) analyses have been performed with urine samples for human and body fluid identification, respectively [2,3]. However to our knowledge no microbiota analysis has been performed with urine samples for aforementioned applications. Previous urological studies on small sample sizes have shown that the microbial composition of urine is highly variable between individuals [4,5]. Therefore the aim of this study is to analyze the inter-individual variability of microbial communities in urine samples and evaluate the discrimination power of urine microbial variation for human identification and body fluid confirmation. Urine samples were collected from 100 Estonian volunteers in the age range of 25-50 (male female ratio of 1:3). The amplification of 16S rRNA hypervariable regions V3-V4 was followed by paired end 250 bp sequencing on Illumina platform. Data analysis is done by using BionMeta algorithms. The analysis of urine microbiota using massively parallel sequencing technologies might provide a valuable tool for human identification.

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60. VALIDATION AND IMPLEMENTATION OF MPS ANALYSIS FOR THE MTDNA CONTROL REGION

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Analysis of the mtDNA in forensic casework is conventionally performed by Sanger Sequencing. Although this method has been the golden standard for analysis of single source DNA samples, mixed DNA samples could be addressed more effectively by Massively Parallel Sequencing (MPS).
We validated a redesigned version of the 'Mitominis' multiplex [1] for the analysis of the mtDNA Control Region optimised for analysis by MPS on the MiSeq® sequencer. Besides numerous single-source samples,four two- and one three-person mixtures were analysed, each in six different ratios (with minor contributions of 1–50%) and in high-template and low-template reactions. In addition, the PCR performance and sequence data quality was investigated by analysing several reference and degraded samples (cemetery) with various DNA input amounts in the PCR (0.1pg – 2 ng).
Data analysis was performed using FDSTools [2] and a series of 228 samples, including samples with C-stretches varying in length from 7 to 13 nucleotides, were analysed to create a reference database for correction of PCR and sequencing artefacts.
A workflow of FDSTools analysis combined with macro-fed Excel® sheets for summarisation of sample results and comparison of mixtures to references was established. The total dataset was used to determine analysis thresholds and to establish interpretation guidelines for casework reporting.

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Topic 02: Non-Human, Microbiome

61. A MOLECULAR ANALYSIS OF THE ARMADILLO *DASYPUS NOVEMCINCTUS* (MAMMALIA: DASYPODIDAE), ONE OF THE MOST COMMON VICTIM OF POACHING IN AMERICA

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One of the taxa suffering from intense poaching activities along its entire occurrence area, Dasypus represents a genus of armadillos that comprises seven living species: D. hybridus, D. kappleri, D. novemcinctus, D. pilosus, D. sabanicola, D. septemcinctus, and D. yepesi. The aim of this study is to analyze the genetic diversity of D. novemcinctus in order to build a haplotype database for molecular identification purposes. Molecular analyses were performed using the database which includes 99 sequences with 762bp of the mitochondrial gene Cytochrome b of D. novemcinctus from several localities including North, Central and South America. We porformed a maximum likelihood analysis and constructed a median-joining network using the Geneious and PopART softwares respectively. Sequences of the remaining Dasypus species and other armadillos (Cabassous tatouay, Chaetophractus villosus, Chlamyphorus truncatus, Euphractus sexcinctus) were also included for the molecular analyses. The obtained results suggest four distinct lineages (groups I, II, III and IV) among D. novemcinctus haplotypes. Group I was the largest, with 53 haplotypes and spread over Brazil, Paraguay and Peru. D. novemcinctus has questionable occurrences and its distribution range needs to be reconsidered. All analyzes reinforce the need for a taxonomic revision for this group in order to elucidate the hiatus in the validity and distribution of some species. Finally, the results reinforced the use of the genetic marker Cytochrome b as an effective tool in the identification of D. novemcinctus.

62. ANALYSIS OF COMPLETE MITOCHONDRIAL DNA OF A FORENSIC IMPORTANT MUSCOIDEA

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Muscidae specimens on the corpses are critically important for forensic investigation for determination of post-mortem intervals and estimation of the time of death. In this study, the complete mitochondrial genome sequenced and analyzed from a Muscidae, *Muscina angustifrons* which have been collected from shade, forest, Mt. Gaewun, Seoul. This is the third complete mitogenome record from the genus Muscina and the first genus record from South Korea. The size of the mitochondrial genome is 16,316 bp and it has typical gene order and gene orientation with ancestral insect genome. It consists of 13

protein coding genes, two rRNA genes, 22 tRNA genes and one putative control region likewise the other records. The main difference in the mitogenome among Muscina records is the length of the putative control region. It has 1,369 bp length putative control region which located between 12S rRNA and tRNA-Ile. There are seven overlapping regions in the genome with 1 to 40 bp lengths. The largest overlapping region is located between tRNA-Leu and 16S rRNA gene. The mitogenome shows 26 intergenic sequences varying from 1 to 110 bp in lengths. The longest intergenic sequence is between tRNA-Glu and tRNA-Phe. Although Muscidae flies are important insects for the forensic entomology, it is hard to identify specimens by morphological examination because of highly similar morphological appearance according to metamorphic stages. Therefore complete mitochondrial genome sequences can be important tool in forensic entomology for identifying species.

63. BRAZILIAN MARMOSETS' POST MORTEM TISSUES TESTED FOR BARCODING (COI)

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Brazil has six different native marmosets (Callithrix) species and some suffer from constant threats inside their territories. Among these threats are crimes of biopiracy, mistreatment and illegal animal trade. The use of DNA barcoding is an important tool to rapdly identify the species' level of biodiversity what is extremely useful to facilitate the efforts for conservation and forensic elucidations. One of the most commonly used locus for this purpose is the Cytochrome C oxidase subunit I (COI) which has many advantages for accurate taxonomic identification. For this study two sample of tooth, hair, skin, muscle and nail remains were collected from 4 individuals of the genus *Callithrix* to test the efficiency of DNA extraction, amplification and sequencing of post mortem bio-material. After extraction, the DNA was quantified, mtDNA (COI) was amplified with a PCR kit, and then sequenced by capillary electrophoresis. Positive results were analyzed by computational software to evaluate the quality of the sequences. Hair and nail were the most difficult tissues to extract good amounts of DNA for successful sequencing, other samples however resulted in good sequences. Our results showed that barcode from softer marmoset tissues were successful and must be chosen, if possible, when identification for forensic purposes is required from dead animals.

64. COMPARISON OF SANGER AND NEXT GENERATION SEQUENCING METHODS FOR APPLICATION OF FORENSIC MICROBIOLOGY

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Forensic microbiology is widely applied to identify people at crime scenes, investigate bioterrorism

events, and determine the cause and time of deaths, by examination of trace evidence. In particular, identification of bacteria, which could be the cause of death, from postmortem specimens is important, because infection with clinically significant bacteria may result in fatal outcomes, such as sepsis or meningitis. The MicroSeq 500 16S rDNA microbial identification system (MSId) is a molecular technique for profiling of bacterial species isolated by microbiology culture methods, based on Sanger sequencing of the bacterial 16S rDNA. The MiSeq Illumina sequencing platform uses next generation sequencing (NGS) for metagenomic analysis of 16S rDNA and culture-independent identification of bacteria. In this study, we analyzed 65 postmortem specimens by both MSId and MiSeq methods and compared the workflows involved with regard to methodology, time-efficiency, cost-efficiency, and performance to determine the limitations and benefits of these techniques for forensic application. We found that MiSeq is more time-efficient, and exhibits superior cost-efficiency when more than 30 samples are analyzed. In addition, MiSeq has a number of other advantages over MSId, including a simpler identification of bacteria that are difficult to culture and use of a larger library of 16S rDNA, allowing more precise identification of bacterial species.

Keywords: Forensic microbiology, Forensic application, MicroSeq 500 16S rDNA microbial identification system (MSId), MiSeq, 16S rDNA

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65. DEVELOPMENT OF SSR AND SNP MARKERS IN OPIUM POPPY BY RAD-SEQUENCING

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The opium poppy (Papaver somniferum L.) is the main source of the drugs like opium and heroin. The species identification is the key method for forensic analysis. However, the lack of highly specific molecular markers is still a significant problem for identification. We sequenced the genomic DNA of opium poppy using restriction-site associated DNA sequencing (RAD-sequencing) and examined the DNA sequences for SSRs and SNPs. A total of 246.14Mb poppy genome fragments, which obtained by restriction endonuclease digestion EcoRI (GAATTC), was sequenced to over 270-fold coverage (68.70Gb) via Illumina sequencing technology. Approximately all of the reads (96.53 %) were assembled into 237.59 Mb of the opium poppy genome. With the length distribution >12bp and the exclusion of mononucleotide repeats a total of 23,925 non-redundant SSRs were identified. Dinucleotide and trinucleotide repeats were the most abundant SSR repeats, accounting for 17.40% and 76.60% of all SSRs. The AAT/TTA repeat was the most abundant trinucleotide repeat, representing 12.64% of trinucleotide repeats. Other SSR repeat types were AT-rich. Moreover, a total of 70,054 SNPs were identified, which contained 57134 heterozygous SNPs and 12920 homozygous SNPs. This is the first study of the development of genomic SSR and SNP markers in opium poppy with RAD-sequencing. The markers developed in this study will be appropriate to address the difficulty derived from the deficiency of markers in opium poppy, which will provide sufficient molecular marker technology for the investigation and judicial determination of opium poppy illicit cultivation and transportation cases.

66. DNA BARCODING STUDY AND APPLICATION OF ANIMAL SPECIES IN KOREA

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National Institute of Biological Resources (NIBR) belonged to Ministry of Environment launched a DNA Barcoding project for Korean indigenous species in 2011. DNA barcoding provides an efficient method for species-level identifications using short DNA fragments. In this respect, 'DNA barcode system for Korean animal species' project aims to develop a standardized, rapid and inexpensive species identification method accessible to non-specialists. Until 2016, total 29,218 animal species were reported in Korea, and about 3,015 species, across invertebrate including insects and vertebrates, were analyzed using mitochondrial *COI* region. In some case, other alternative markers were used such as *CYTB*, *MSH1*, *16S rRNA* and *12S rRNA* depending on taxon. We had several practical applications of species identification using DNA barcoding: 1) for confiscated leathers and snakes, 2) for CITES species such as rhino horn and whales 3) for investigations of illegal import of endangered species 4) for investigations of origin violation of fisheries 5) for investigation of illegal distribution of wild animal 6) for strange substances in fermented food, 7) for specimens from 313 cases of birdstrike, 8) for research of micro-fauna, 9) for unknown feathers or hairs from wild animals, and so on. DNA barcoding approach has great potential in aiding of species identification, particularly in regions of the world that lack the vast research collections and individual expertise for morphologic identifications. It is considered as a useful tool for management and conservation of wildlife.

67. DNA-BASED DETECTION OF WHEAT IN FOOD SAMPLES AND GASTRIC CONTENT

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Background: The sensitive detection and specific identification of plant material can be a powerful tool in forensic casework. As example, the identification of an allergen in ingested food samples could clarify the cause of death in otherwise unclear casualties. Methods: The applicability of previously described PCR assays, detecting wheat-specific sequences on the internal transcribed spacer region [1], was evaluated. A SYBR Green based real time PCR procedure with subsequent melting curve analysis was established using the original primer sets. DNA extracted from raw flour was used as positive control. DNA extracted from 34 samples of vegetal food and meat from various species was studied. Furthermore, DNA extracted from the gastric content of eleven forensic autopsy cases was investigated. The stage of digestion in these samples was graded as previously reported [2]. Residuals of bread or pasta had been macroscopically described in the corresponding materials. Results: Positive results were obtained from raw powder and wheat processed to standard food components such as bread or pasta. No unspecific amplification results were observed with DNA from raw meat samples. Only one single specimen of commercially prepared minced meat showed positive amplification. None of the plant samples showed a positive reaction, with the exception of specimens from soybean sprouts or commercially prepared mixed salads. Seven of eleven PCRs of stomach contents showed positive amplification results. Conclusions: Sensitive detection of wheat in ingested food can be obtained employing these assays. Contaminations along with food processing might explain the detection of

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68. FORENSIC METAGENOMICS: SIMULTANEOUS DETECTION OF DIVERSE TAXA USING MASSIVELY PARALLEL SEQUENCING (MPS)

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Maximising the information obtained from forensic samples provides additional intelligence to aid identification of the criminal perpetrator. Investigative intelligence tools deployed when standard identification methods have been unsuccessful include biogeographic ancestry prediction and the inference of phenotypic traits such as hair and eye colour. Microorganisms reside in and on human tissues and body fluids and inter and intra individual variation in the microbiota within these locations has been observed. On touching an item a two-way transfer of material occurs, the depositor's taxonomic profile will be transferred to the item while taxa housed on the item will be transferred to the surface of the depositor's skin. The taxonomic profile recovered from the item will be admixed with things that the individual has come into contact with up until the deposition of that sample and can therefore provide additional intelligence - do they have a dog? What breed? This method moves away from sequencing specific microbial regions (e.g. 16S for bacteria) and towards simultaneously sequencing bacteria, fungi, viruses, archaea and protozoa along with human and animal DNA. This method was tested and validated by homogensing saliva collected from 20 individuals and later using the ZymoBIOMICS™ Microbial Community Standard (Zymo Research, USA). Samples were either extracted or directly amplified using a random and then specific priming strategy. Amplified samples underwent quantification and library preparation before MPS using the Illumina® MiSeq®. Sequence data was processed using an in-house bioinformatics pipeline and data analysis and computational graphics performed with MEGAN6 (University of Tübingen, Germany).

69. FORENSIC PARAMETERS FOR ELEVEN STRS IN ANGUS BRANGUS BREED FROM NORTHWEST COLOMBIA

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Livestock (Bos taurus) is one of the main sources of protein in the human diet, and is one of the major commercial activities worldwide involving billions of dollars annually; Molecular genetics has been

involved for decades in the study of this species. Animal genetic analysis has been used for various objectives in the past, including kinship analysis, lineage reconstruction, pedigree records, population genetics, and lately to involve evidence of animal origin in forensic casework, to clarify cases of cattle theft or to certify genetically high value products.
In the present work, 339 bovines of the Angus-Brangus breed were typified from the filiation and animal registration service of the IdentiGEN laboratory of the University of Antioquia. DNA samples were obtained from hairs extracted by Chelex 10%. DNA was quantified by spectrophotometry and diluted to 2 ng/ul concentration. Eleven STRs included on Applied Biosystems stockmarks kit were used according to the manufacturer's recommendations; amplicons were separated on ABI3130 genetic analyzer, and sized by genemapper V3.2 software. Genotypes were used to calculate allele frequencies and forensic parameters using Powerstats software (Promega Co.) that ranged from: observed heterozygosity 0.6777-0.8869 (TGLA53-TGLA127), probability of coincidence 0,1442-0,0486 (TGLA122-TGLA127), power of discrimination 0,9513-0,8557 (TGLA122-TGLA127), PIC 0.6379-0.8325 (TGLA122-TGLA127) probability of exclusion 0,3321-0,7687 (TGLA122-TGLA127), typical paternity index 1,3617-4,4210 (TGLA122-TGLA127). In this sample the highest efficiency markers based on the above parameters were in order (descendent): TGLA127, BM2113, ETH225, INRA23, TGLA53, ETH010, ETH3, SPS115, TGLA126, BM1824, TGLA122. Arlequin and GenAlex software were used to evaluate HWE by exact test.

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70. GENETIC DIVERSITY OF GEOPHAGUS BRASILIENSIS FROM THE SOUTH AMERICAN ATLANTIC RAINFOREST

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The Atlantic Rainforest is one of the global conservation hotspots, representing a seriously threatened area with high levels of endemism and species richness. The fishes from the Atlantic Rainforest are one of the most exploited vertebrate groups by the international aquarium trade, an industry with an annual trade volume of about U$15-25 billions, now aggravated by the Internet e-Commerce and playing a crucial role on the sales and distribution of these species. The Cichlidae is one of the most exploited groups with several species been commonly sold in several online sites and aquarium stores all around the world. In this sense, our aim is to study the genetic diversity of the native cichlid Geophagus brasiliensis from the South American Atlantic Rainforest in Brazil. We constructed a database including 75 sequences from Geophagus brasiliensis from the Ubatiba river with 540bp of the D-Loop region using the primers H16498 and L15774M and conducted the molecular analyses with the softwares Geneious v4.82, DnaSP v5, Arlequin v3.5 and PopART. Our results point that although G. brasiliensis copuld be considered as a territorialist species which exhibits parental care and small movement rates, a populational structure could not be observed along the sample area, indirectly suggesting the existence of considerable gene flow at least among the sampled populations.

71. HEPTAPLEX-DIRECT PCR ASSAY FOR SIMULTANEOUS DETECTION OF FOODBORNE PATHOGEN

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Foodborne pathogens pose significant problems for public health and economy. Conventional bacterial identification relies on cultivation, which is time-consuming and costly. In this study, we aimed to develop and validate a heptaplex-direct PCR assay for simultaneous detection of seven foodborne pathogens without DNA extraction and enrichment. Seven primer pairs were used to amplify the virulent genes of target strains and found that the assay provided the expected PCR fragments of 583, 490, 415, 343, 224, 209 and 105 bp for *Shigella* spp., Shiga toxin-producing *Escherichia coli* (STEC), *Streptococcus pyogenes*, *Campylobacter jejuni*, *Salmonella* Typhi, Listeria *monocytogenes*, and *Staphylococcus aureus*, respectively. The assay was highly reproducible and specific when tested with nine other strains commonly found in food. The limit of detection was 10^6-10^0 CFU/ml. Moreover, the assay was applied to 22 and 100 artificially- and naturally-contaminated food samples and provided a statistically equivalent efficiency to the culture method. The study thus indicates that the heptaplex-direct PCR assay can be used is in microbial forensic science and medical diagnoses, especially in food investigation.

72. KBC HORSEFILING, A NOVEL KIT FOR HORSE PROFILING

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The wide range of applications of horse genotyping in parentage testing, population genetics, diversity studies and forensic investigations has made it a valuable method. In order to achieve an accurate equine DNA genotyping, we have developed a multiplex PCR kit (KBC HorseFiling) encompassing 22 Short Tandem Repeat (STR) markers (HTG7, HMS5, VHL20, CA425, HTG10, HMS3, KBC51, ASB17, AHT5, HMS2, AHT4, KBC61, HTG6, ASB2, LEX3, HTG4, ASB23, HMS7, ASB25, HMS6, HMS1 and KBC71). 5 markers (HMS5, KBC51, KBC61, ASB25, and KBC71) have been added to common 17 markers located in 4 other chromosomes of equine of available kits in order to increase the power of discrimination. Despite all primers of these 22 markers are novel and were designed such a way that reduce allele drop-out, results are fully compatible with that obtained from other available kits by comparing 100 samples in parallel tests.
The capability of direct amplification of any DNA source such as saliva, hair root, bone, and blood on DNA banking cards, makes the KBC HorseFiling a powerful and rapid kit in DNA genotyping. Individuals usually send horse's hairs for testing. By using KBC HorseFiling kit, the DNA extraction step is not needed and only 1 hair root is sufficient for accurate results.
In addition to precise horse parentage testing, KBC HorseFiling can be applied to distinguish between commingled samples containing human and animal tissues in forensic cases.

73. MAPPING OF TIMBER RATTLESNAKES (*CROTALUS HORRIDUS*) – PHYLOGEOGRAPHIC ANALYSIS USING THE MITOCHONDRIAL DNA CONTROL REGION

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Timber Rattlesnakes (Crotalus horridus) are an endangered, threatened or candidate species in the eastern region of North America and therefore protected by law. Loss and destruction of habitat and intentional killing have decreased the number of individuals of this slow reproducing species over the last several decades. In an effort to monitor their population development, the phylogeographic origin and classification of subpopulation was examined. Studies from Bushar et al. (1998, 2001) and Villarreal et al. (1996) identified three major population groups partially in Pennsylvania, New Jersey and Virgina: the Appalachian Plateau population, the Ridge and Valley and the Coastal Plain. The development of an assay for rapid identification of the geographical origin of C. horridus individuals would provide a powerful tool to aid the development of conservations plans and combat against poaching of rattlesnakes and their illegal skin and pet trade. Therefore, mitochondrial control regions of 30 Timber Rattlesnakes (11 Appalachian Plateau, 11 Ridge and Valley, 8 Coastal Plain) were sequenced to identify potential single nucleotide polymorphisms correlating to the geographical origin of an individual. Four SNPs could be identified, for the rest, both control regions showed no interindividual variation. However, these polymorphisms showed no correspondence to geographical origins, as did grouping in phylogenetic analysis. Future studies will extend the sample size to allow better assessment of the results and add statistical significance. Furthermore, populations from other areas of North America will be included to evaluate possible phylogeographic distinguishable population in the whole North American habitat of C. horridus.

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74. MICROBIAL FORENSIC ANALYSIS OF HUMAN-ASSOCIATED SKIN BACTERIA

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Forensic science involves any descriptive sciences that aim to provide important evidences in court. Microorganisms are microscopic organisms, either single-celled or multicellular. The characterization of

microbial community compositions and distributions can compensate for the limitations of conventional forensic investigation in personal identification. As the largest organ of the human body and primary interface with outer environment, skin harbors various microorganisms. A recent study recognized the significance of bacterial flora found on the surface of the skin and its application in personal identification.1) In particular, hand surface can be an environment of primary interest, as it harbors greatest microbial diversity.2) Well known bacterial genera in human hands include Propionibacterium, Streptococcus, Staphylococcus, Corynebacterium and Lactobacillus.3) In this study, we explored the diversity of microbial communities inhabiting the palms of different individuals using culture-based and culture-independent methods with 16S rRNA gene and 7 house-keeping genes. Bacterial strains from the palms of seven participants were isolated using culture-based method. Among the isolates, about 15% were identified as Staphylococcus epidermidis that occurs as one of the main normal flora in skin, and were analyzed by multilocus sequence typing (MLST). The S. epidermidis strains were classified into 5 known and 6 new sequence types, where 4 new allele types were also discovered. The DNA samples directly obtained from the hands were subjected to the terminal-restriction enzyme fragment length polymorphism (T-RFLP) analysis with two restriction enzymes. The resultant data confirmed a high potential of hand microbial flora in the application for personal identification.

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75. MITOGENOME PROTEINS BASED PHYLOGENETIC STUDIES OF DIPHTERA WITH THREE NEW MUSCOIDEA RECORDS

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Forensic Entomology is the use of the insects, and their arthropod relatives that inhabit decomposing remains to support criminal investigations. For this support the identification of specimens found on investigation is extremely important. However, it is hard to identify specimens by morphological examination because of highly similar morphological appearance. In this study, mitochondrial genes based phylogenetic tree of the Diptera reconstructed. For reconstruction, the complete mitochondrial genomes of the species were retrieved from the GenBank and amino acid sequences of all protein coding genes except ATP8 gene were used for analysis. In total 97 species of 14 different families were used. Within these 97 species 94 of them were retrieved from GenBank and three new Muscoidea records were added. In the study, mainly we focused on forensic important families which are Calliphoridae, Sacrophagidae and Muscoidae. Within these families, Calliphoridae has 24 species records and it is the only monophyletic group. Besides it has sister group relationship with Sacrophagidae. However Sacrophagidae is a paraphyletic group and one member of the family is early branched than Calliphoridae family. On the other hand Musicdae is presented by seven species in which three of them are recently recorded. Musicdae is the earliest branched family and also it is a paraphyletic group. Even the most commonly DNA-based specimen identification is the COI based

barcoding; it has misidentification risk because of pseudogenes. To overcome these risk complete mitochondrial genome based identification could be a useful tool for forensic entomology.

76. MOLECULAR AGE MARKERS FOR BLOWFLY PUPAE: SPECIFICITY AND ROBUSTNESS AFTER SPECIMEN STORAGE

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Age estimation of juvenile blow flies is a common method for PMI estimation within Forensic Entomology when classical parameters (Rigor Mortis, Livores etc.) are no longer pertinent. Blow flies are attracted by corpses which serve as oviposition site already shortly after death. Therefore, the age of the developing immature stages and the minimal PMI (minPMI) are strongly correlated. Entomological minPMI estimation is well established for blow fly larvae using their age dependent length. However, this character is not applicable when specimens are collected after the process of pupariation. Therefore, we focus on the intra-puparial period = the metamorphosis via gene expression analysis.
Using MACE to identify age specific transcripts of the intra-puparial stages of the blow fly Calliphora vicina we have identified several transcripts suitable as precise age marker because they are up-regulated for only a specific day during metamorphosis. The markers and the established qPCRs will be presented.
mRNA can be highly vulnerable to degradation, so we also tested if a storage of the sampled specimens affects the qPCR results. To mimic a field scenario where insects are sampled and stored without access to conservation fluids or freezers we stored specimens in 70% Ethanol at room temperature up to several weeks.
Up to now we tested two reference genes and it can be stated that the use of day specific age markers is possible. Hence, the current results suggest that storage at RT in 70% Ethanol does not seem to affect transcript quantification of intrapuparial stages.

77. MOLECULAR IDENTIFICATION OF A REEF FISH COMMUNITY FROM THE BAÍA DE TODOS OS SANTOS, BAHIA, BRAZIL

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The reef fish community is one of the most explored fish stock by the local and medium scale fishing activities as well as by the international ornamental fish trade. Despite of these intense commercial activities, several reef species are poorly known. The reef fish community limited knowledge makes difficult the commercial regulation and also the adoption of conservation policies and general law enforcement. The present study deals with the molecular identification of a reef fish community from the Baía de Todos os Santos, Bahia, Brazil in order to construct a reference database for molecular identification of these species. We evaluated the molecular identification of 51 species included in 28 distinct families. The specimens were caught using freediving techniques and tissue samples were obtained from the epaxial musculature. The DNA barcoding methodology was used for the amplification of a 650bp fragment from the mitochondrial COI gene. Among the 51 obtained COI sequences, 16 of them represent new register for the species in the Brazilian coastal region including two species (Gramma brasiliensis and Scarus trispinosus) without any molecular sequence available until now. We also identified some overexploited species such as the case of Lutjanus cyanopterus and S. trispinosus, both included in the IUCN Red List. Finally, when compared to previously available sequences from NCBI and BOLD databases, the K2P distances observed (higher than 9%) suggest the existence of unidentified or cryptic species within this community.

78. MULTIPLEX PCR IN NON-HUMAN DNA MOLECULAR IDENTIFICATION OF *ASCARIS SPP.* IN FORENSIC BIOLOGY

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Forensic Parasitology is a promising new tool to investigate and prevent illegal trade in protected species along with human public health protection. It could provide previously overlooked key evidence for specific circumstances based on the presence of parasites in forensic cases. Several species have been reported to have Ascaris worm infections (pigs, humans, rabbits, guinea-pigs, mice, wild-boars, goats, antelopes). DNA parasite profile extracted from endangered animals could help determine specific geographical movements (wild/captive-bred) and finding possible vectors and zoonosis. Epidemiology also could be studied in deaths cases for Ascaris infestations. Interestingly, problems of people claiming a certain nationality with no documentation could be feasibly solved by tracing down the genotype origin of host-dependent organisms. We present 2 multiplex-PCR systems (7STRs each one) which were designed based on PCR-monoplex previous reports. Some primers were modified in order to optimize amplicon size and to homogenize annealing temperature. DNA was extracted by Phenol-Chloroform-Isoamyl-Alcohol from pig Ascaris worms. PCR reaction was 1X-QIAGEN®-Multiplex-PCR-Kit; thermal profile: 95°C(15min); 30 cycles: 94°C(45sec), 54.8°C(60sec) and 72°C(75sec); final elongation at 72°C(1hour). Both set of primers (0,45-4uM) and DNA(5ng/uL) were regulated, successfully decreasing artifacts. Finally, amplicons were separated by CE in ABI3130 and sized by Gene-Mapper IDv3.2 software, panels and bins were constructed. Genetic profiles were highly encouraging, showing amplicons consistent with expected allelic ranges and adecuated heterozygotes balance that allowed genotyping of Ascaris spp. Reduction in both processing time and reagents was evidenced. We are currently investigating a larger sample to determine genetic diversity and forensic parameters for Ascaris spp.

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79. OPERATION TIGER´S EYE: DNA TESTING OF TRADITIONAL CHINESE MEDICINE ARTIFACTS IN CZECH REPUBLIC

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Traditional Chinese medicine (TCM) has been practiced for thousands of years, but only within the last few decades has its use become more widespread outside of Asia. Concerns continue to be raised about the efficacy, legality, and safety of many popular complementary alternative medicines, including TCMs. Ingredients of some TCMs are known to include derivatives of endangered, trade-restricted species of plants and animals, and therefore contravene the Convention on International Trade in Endangered Species (CITES) legislation. Some recent textbooks of TCM still recommend formulas containing various animal tissues such as tiger bones, antelope, buffalo or rhino horns, deer antlers, testicles and os penis of the dog, bear or snake bile. Usually, animal tissues are combined with medical herbs.
The authors will present the DNA based species identification results obtained from various seized materials during the operation Tiger´s Eye run by Czech law enforcement agencies. Some of the artefacts submitted for DNA analysis did not yield any animal DNA or no amplifiable DNA at all. The methods employed for the analyses comprised sequencing of animal mtDNA genes coi and cytb. However Sanger sequencing would not be appropriate method for a complex mixtures of biological material, where more different animal or even plant species are used for the preparation of the particular TMC. DNA analysis of complex mixtures would thus require the use of Massive parallel sequencing (MPS)

80. POTENTIAL USEFULNESS OF SNP IN THE 16S RRNA GENE SERVING AS INFORMATIVE MICROBIAL MARKER FOR FORENSIC ATTRIBUTION

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The field of microbial forensics focuses on the attribution of numerous microbes used by perpetrators in biocrime and bioattack, including many foodborne pathogens such as Escherichia coli O157:H7 caused

serious zoonotic disease. Recent source tracing analysis with the 16S rRNA gene, even genome-wide variations of a pathogen, is increasing as sequencing technologies advance. By using whole genome sequence analysis, more SNPs have been used to create a very finely resolved phylogenetic tree for discriminating close evolutionary relationship among strains. In our study, 1401bp of 16S rRNA gene in Enterohemorrhagic Escherichia coli (EHEC) O157:H7 strains were sequenced. The result showed that nucleotide transition and nucleotide transversion happened at 14 positions of sequence in the research strains, and variation of seven positions was located at one of nine separate hypervariable regions (V7). Another SNP within the constant region of 16S rRNA gene was also observed as the previous studies. Through comparing the available sequences obtained from the NCBI database, SNP was the only 16S rRNA polymorphism detected among the closely related EHEC O157:H7 stains. Our investigation demonstrates that unique SNPs in 16S rRNA gene have potential discriminatory power as other species-/strain-specific informative markers for attribution purpose.

81. PROBING THE POTENTIAL OF THE SHARK PANEL INDEL MULTIPLEX V2.0 ON THE FORENSIC IDENTIFICATION OF BATOID ELASMOBRANCHS

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The Elasmobranchii comprises the diverse and important group of sharks and rays. The group includes some of the ocean's largest predatory fishes, suffering unsustainable fishing activities and is commercially overexploited for their meat and fins. Overfishing has resulted in significant population declines and several 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 have provided important information about these species, allowing the management of natural stocks and preventing their decline. Population genetics and connectivity data 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 and rays in a forensic context. Following the development of the Shark Panel v1.0 which includes uniquely sharks, now we tested the effectiveness of the v2.0 multiplex on batoid elasmobranchs. We carried out a systematic molecular analysis using 85 previously published mitochondrial 16S rRNA gene sequences obtained from the NCBI database and found that indels in the 16S rRNA gene can be used to distinguish several analyzed 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 elasmobranchs identification in a forensic context.

82. RAPID CLASSIFICATION OF UNKNOWN BIOLOGICAL MATERIAL USING NOVEL TRIPLEX ASSAY

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DNA laboratory of Forensic DNA Service, Czech Republic regularly tests traditional Chinese medicines seized by Czech customs officials, Czech Police and The Czech Environmental Inspectorate. The seized artefacts include powders, jelly substances, osteological artifacts (hair, teeth, claws,…), liquids with macerated pieces of biological material and tinctures. The methods employed for the species identification of plant and animal material include DNA extraction, DNA quantification and Sanger sequencing of selected mitochondrial or plastid regions. Samples without clear origin are tested using laboratory developed Triplex PCR assay for rapid classification. The assay enables to detect the presence of animal/human, plant DNA and to detect the presence of PCR inhibitors. The target genes are plant rbcL gene (RuBisCO), animal/human coi gene (cytochrome oxidase I) and a synthetic oligonucleotide sequence IPC for plant, animal and Internal Positive Control (IPC) measurement, respectively. The size of the amplicons for the three targets is 285 bp for rbcL, 511 bp for coi and 702 bp for IPC. The minimum amount of DNA detected by this Triplex PCR assay is 50 pg for rbcL gene and 50-100 pg for coi gene. PCR product can be further used for subsequent sequencing reactions.

83. SIMULTANEOUS SPECIES IDENTIFICATION IN MILK AND DAIRY PRODUCTS USING DIRECT PCR

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Fraudulent milks and dairy products cause health and economic problems. As such, these acts are considered illegal worldwide. Forensic science can play an important role in helping to identify species in milk and dairy products to help enforce national legislations regarding these issues. In this study, we successfully developed and validated a triplex direct-PCR assay with capillary electrophoresis detection to identify the three common milk species: cow (Bos taurus), sheep (Ovis aries) and goat (Capra hircus). The assay amplified mitochondrial COI and cyt b genes and generated PCR products of 93, 173 and 231 bp for cow, sheep and goat, respectively. The assay was highly reproducible, specific to target species, sensitive, and showed 100% identification accuracy. Additionally, it was applicable to milk and its dairy product samples. The study suggested that the developed technique can be used for real case sample analysis for food safety and law enforcement for consumer protection.

84. STROE DEER: A NEW MULTIPLEX PCR TO GENOTYPE ROE DEER, DEVELOPED ACCORDING TO THE ISFG RECOMMENDATIONS REGARDING THE USE OF NON-HUMAN DNA

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Interest in forensic use of animal DNA has increased in recent years. Apart from taxonomic classification of unidentified animal material, there has been a rise in demand to assign samples to a particular individual. Poaching, illegal wildlife trade and hit-and-run accidents involving wildlife are examples where genetic individual assignment can be useful. Despite this wide range of applications, the availability of forensically validated methods is limited to a few species. After several requests to individually genotype roe deer, *Capreolus capreolus*, we decided to develop a species-specific STR multiplex PCR (STRoe deer), following the ISFG recommendations regarding the use of non-human (animal) DNA. STRoe deer contains 13 tetrameric STRs and two sex-specific markers. Based on 513 roe deer samples collected from 8 cantons (out of 26) in Switzerland, we validated the method and calculated population genetic parameters. Probability of Identity was 4.8×10^{-13} ($PI_{Sibs} = 1.3 \times 10^{-5}$), no substructure was found in the dataset and the inbreeding coefficient was low ($F_{is} = 0.0275$). On the basis of a mock case, we will demonstrate the functionality and benefits of STRoe deer.

85. THE AMPLIFICATION OF THE MITOCHONDRIAL GENOME OF THE ENDANGERED BUFFY-TUFTED-EAR MARMOSET *CALLITHRIX AURITA* (PRIMATES: CEBIDAE) FOR MASSIVE PARALLEL SEQUENCING USING THE HISEQ 2500 PLATFORM

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Callithrix aurita is a native small primate species from the Brazilian Southeastern Atlantic mountains. Considered as vulnerable and figuring the IUCN Red List, the species currently struggles with a reduced population size and faces several obstacles such as congeneric competition and the obvious anthropic pressure. Here we describe our amplification strategy to obtain high quality DNA of the endangered marmoset species Callithrix aurita from the South American Atlantic Rainforest, for use on massive parallel sequencing with the HiSeq 2500 platform. Two sets of primers were designed for the amplification of two overlapping fragments from the mitochondrial genome of Callithrix aurita. The cycling conditions were as follows: 94ºC for 2 min, 30 cycles of 94ºC for 30 s, 59ºC for 30 s, 68ºC for 10 min, 72ºC for 7 min, 4ºC forever. Both reactions were performed using the La-Taq Clontech/TAKARA Long PCR Kit. Two fragments of about 9Kb were successfully amplified comprising the fragments F1 165-9229 and F2 8531-639 of the mitochondrial genome of the Callithrix aurita. The quantification of the DNA was made using the Qubit fluorimeter and the fragments are now under sample preparation and sequencing using the Nextera DNA Kit within the HiSeq 2500 platform.

86. THE QUANTITATIVE ANALYSIS FOR EPINECROTIC BACTERIA IN THE RECTUM OF INDOOR CADAVERS BY USING RT-QPCR

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Postmortem interval (PMI) estimation is one of the largest challenges for forensic investigation. Traditional methods of PMI estimation has been always hindered by cadaver decomposition, which mainly caused by microorganisms and insects. Previous studies have shown that epinecrotic bacteria plays an important role in cadaver decomposition. In the present study, the microorganism communities in the rectum of rat cadavers decomposed without sarcosaphagous insects interference were taken every day until the sample sites were corrupted. The relationship between the relative abundance of main epinecrotic bacteria genera and PMI were analyzed by real-time fluorescent quantitative Polymerase Chain Reaction (RT-qPCR). RT-qPCR has high efficiency and sensibility in bacterial quantitative detection and could ascertain the trend of specific genera variation in the decomposition process. With the simple operation and low-cost material, it could be applied directly in forensic practices. In our results, some bacterial genera like the Bacteroides decreased gradually with the PMI extension ($P<0.05$). The regression equation between the relative abundance Bacteroides and PMI were obtained and could been used to establish a quantitative method for PMI estimation potentially.

87. TUNA FISH IDENTIFICATION USING MTDNA MARKERS

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Included in one of the most commercially valuable fishes traded all around the world, the genus Thunnus is a Scombridae which comprises eight nominal species commonly known as tunas. The most widely traded in the international fish market are the species known as Atlantic bluefin tuna (Thunnus thynnus), bigeye tuna, (Thunnus obesus), Southern bluefin tuna (Thunnus maccoyii), yellowfin tuna (Thunnus albacares), albacore (Thunnus alalunga), and Pacific bluefin tuna (Thunnus orientalis). As expected for congeneric species, they are morphologically very similar and the species identification, especially in its traded forms is difficult. Several protocols have been described for species identification of marine products however, the low genetic distance among the several tuna species commonly confound the obtained results depending on the molecular marker used. Additionally, several authors pointed that the use of the DNA barcoding methodology is not effective and cannot recognize all the species due to lack of resolution of this mitochondrial marker. Here we identified a potential region for species delimitation within the genus Thunnus. The mtDNA region between sites 8092 and 8847 which comprises the mtDNA genes AT8, ATP6, and COX3 presented polymorphic sites which allow precise species delimitation. It also remains with the same vantages of the DNA barcoding region in relation to fragment size (~750bp) and conserved flanking regions for

primer annealing. Flanking primers were developed and are now being tested with samples obtained from the local fish markets and also from industrialized products.

88. THE INTEGRATED WEB PORTAL SYSTEM FOR THE FORENSIC ASSESSMENT OF BIOLOGICAL SPECIES

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Species identification for the forensic assessment of biological species using DNA makers has been widely used by scientists as an effective molecular tool for tracking adulterations in food and for analyzing samples from alleged crime incidents. The curated reference databases that are correctly assigned to taxa with adequate sampling and taxon coverage to fully evaluate both the intraspecific and interspecific variations is of major importance to the assignment of sequences to species. Therefore, we collected and integrated the related information (image, taxonomic information, ecological data, geological location, etc.) from GenBank, BOLD, SILVA and Korean domestic institutes, in order to verify the identification of forensic samples as well as to document potential new species without taxonomic information. We also developed analysis pipelines for easily identifying and analyzing data through the web portal system.

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89. PLANT DNA BARCODING SYSTEM FOR FORENSIC APPILICATION

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Non-human DNA analysis as well as human DNA analysis is growing importance to investigate the crime. Above all, plant evidence that collected in crime scene can be important key to solve the crime; moving of corpse, route tracking of suspect, identifying of narcotic plant, etc. We optimized the plant DNA barcoding system to analyse from slight amount of plant evidence.
We had checked rbcLa, matK, trnL-F and rbcL regions besides psbA-trnH region, and also Dr. Max II Master Mix(MGmed) for amplifying. The analysis results of 13 species of woody plants and 20 species of herbaceous plants, identified earlier, all of woody plants and 5 herbaceous plants were clearly identified its species. 14 herbaceous plants were able to identify its genus and an herbaceous plant was unidentified. We also able to identify 2 plant evidences, identified earlier, from crime scene with modified plant DNA barcoding system.
Plant DNA barcoding system has been already researched in lots of botanical laboratories. In this study, we used 5 regions to increase discrimination accuracy and domestic taq polymerase for cost-cutting

and convenience. As a result, our modified DNA barcoding system is an effective method to analyse slight amount of plant evidence to identify its species and genus.

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Topic 03: Population Genetics, Lineage Markers, Ancestry, X-Chromosome Variation, Paternity

90. 500 YEARS LATER: UNDERSTANDING THE GENETIC ANCESTRY OF THE SOUTHEAST REGION, BRAZIL

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The Southeast region of Brazil is the main economic region, the most populous and with the major cultural and genetic miscegenation of the country. Due to its historical context, it mainly includes descendants of Portuguese and Italians, but also has strong influence of Amerindians, Africans, Spaniards and Germans. The influence of Arabs and Asians is very small. In this work, the ancestral proportions of the population of the Southeast region were evaluated through a genetic data compilation with diverse markers (Y-SNPs, AIM-INDELs, mtDNA and X-INDELs) in individuals from the states of São Paulo, Rio de Janeiro, Minas Gerais and Espírito Santo, which form this region. The proportion of paternal lineages (Y-SNPs) with European origin is much higher (86.57%), being 10.86% of African origin and 1.98% native American. Instead, the proportion of African origin (44.1%) predominates in the maternal lineages (mtDNA), followed by Native American with 29.2%, European with 26.2% and only 0.5% for Asian. With the autosomal AIM-INDELs the ancestral proportion obtained was 59.12% European, 28.12% African and 12.76% Native American. The autosomal chromosomes tend to reflect an average between the ancestry proportions of the Y chromosome and mtDNA. No Asian ancestry was identified with these markers. In preliminary analyzes with X-INDELs in samples from São Paulo, we found the following ancestral proportions: 29.4% African, 49.7% European and 20.9% Native American. This genetic miscegenation occurred since the formation of this region, when Portuguese colonizers mated with native women and later with slaves brought from Africa.

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91. A COMPREHENSIVE POPULATION ANALYSIS ON 15 AUTOSOMAL STR MARKERS IN CHILE

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Previous forensic genetic studies carried out in populations from Chile were carried out on a limited number of STR markers and a discrete geographic coverage. Here, we estimated the allele frequencies for the 15 autosomal STR loci included in the AmpFlSTR1 Identifiler in a sample of 986 unrelated Chileans of non-Native American individuals. The samples represent five different urban locations in Chile: Iquique, Santiago, Concepción, Temuco and Punta Arenas. We provide frequency distributions and forensic parameters for each recruitment site. Analyses were also undertaken by merging the data into five sample locations. No significant statistical differences could be detected between different regions in Chile1. These data represent one of the very few studies performed on autosomal STRs in Chile and therefore provide a useful tool for forensic casework carried out in the country.

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92. ALLELE FREQUENCY AND FORENSIC GENETIC VALUES OF 12 STR LOCI USING THE INVESTIGATOR® HDPLEX KIT

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We genotyped and calculated forensic values for 12 STR loci from 990 individuals of the Korean population using the Investigator® HDplex kit. No significant deviations from HWE (after Bonferroni correction for multiple testing) or genetic linkage disequilibrium were observed. A total of 192 alleles were detected in 12 autosomal STR loci. The most informative locus was SE33 (Hobs = 0.9475), and the least informative locus was D2S1360 (Hobs = 0.5964).The calculated matching probability and power of discrimination ranged from 0.0080 to 0.2014, and 0.7986 to 0.9920, respectively. The combined PD for all 12 loci was 2.6222 × 10-16. We suggest that the markers of the kit are highly informative as corroborative tools in forensic DNA analysis.

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93. ALLELE FREQUENCY DATA OF 20 STR LOCI IN 2000 KOREAN INDIVIDUALS

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Short tandem repeats (STRs) are extensively used for human identity testing applications. Recently, the Korean DNA database management committee announced an expansion of the 13 STR loci to increase discrimination power in forensic human identification. In order to meet this objective of forensics and evaluate expanded STR loci, Supreme Prosecutors' Office (SPO) and National Forensic Service (NFS) examined a Korean population sample of 2000 unrelated individuals. Allele frequencies for 20 STR loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, and D22S1045) available in commercial STR kits were calculated. Statistical analyses were carried out to determine the basic parameters of population genetics. Of the statistics, the power of discrimination values was from 79% to 97%, so that all tested loci can be reliably used to establish a STR DNA database for the Korean population. In addition, the effect of sample size on the diversity of genotypes was analyzed to estimate representative allele frequencies using rarefaction analysis. Allele frequencies of 20 STR loci would be used for forensic personal identification and paternity testing in the Korean population since August 1, 2017.

94. ALLELIC FREQUENCIES OF 15 AUTOSOMAL STRS FROM TWO MAIN POPULATION GROUPS (MAKUA AND CHANGANA) IN MOZAMBIQUE

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Mozambique's population is composed of various ethnic groups from Bantu origin. There are two main ethnic groups, Makua which represents 26% of the population and is predominately found at the north of the country and Changana representing 11% of the population and found in the south. DNA testing for forensic purposes is not a practice in the context of court proceedings in Mozambique. To interpret the significance of a match between genetic profiles from two different individuals, population allelic frequencies at each locus in question must be known. This work aimed to determine the allelic frequencies of 15 autosomal Short Tandem Repeats (STRs), (D8S1779, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA), in 160 samples of unrelated individuals. Statistic parameters of forensic interest were assessed as well as a comparative study with African, European and Latin-American populations. Our data showed no significant differences between Makua and Changana populations ($P>0.05$). No deviation from Hardy-Weinberg equilibrium was observed in all 15 autosomal STR loci ($P>0.05$). Combined power of discrimination and combined probability of exclusion were 0.99999999997 and 0.999999466 respectively. Locus by locus comparison showed significant differences ($P<0.05$) between our population and those from Africa, Europe and South America. Tri-allelic patterns at TPOX locus were found in six samples from two males and four females. We also found allele variant 16.1 at FGA locus in one sample. Our data is a small contribution toward the STRs allele frequencies database of

Mozambique's population for forensic purposes.

95. ANALYSIS OF 16 AUTOSOMAL STR LOCI IN UYGHUR AND KAZAKH POPULATIONS FROM XINJIANG, CHINA

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The NGM SElect™ Kit is a highly robust multiplex kit developed for human identification. This kit contains the expanded European Standard Set of Loci and the highly polymorphic SE33 locus. The high power of discrimination makes it one of the most informative kits for forensic applications. In order to evaluate the kit and get population data for Chinese Uyghurs and Kazakhs, the genetic polymorphisms of 16 autosomal STR loci included in the NGM SElect™ Kit: D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, D10S1248, D22S1045, D2S441, D1S1656 D12S391, SE33, and Amelogenin were studied in 324 unrelated Uyghur individuals and 124 unrelated Kazakh individuals from the Xinjiang Autonomous Region of China. Allele frequencies and forensic relevant parameters were calculated using the Arlequin software and DNAVIEW. The genotype frequency distribution of each locus did not deviate from Hardy-Weinberg Equilibrium in any of two populations. The forensic parameters indicated that the kit is suitable for personal identification, paternity testing, and complex kinship analysis in Uyghur and Kazakh populations. Allele frequency data for the STR loci was compared with other previously published population data.

96. ANALYSIS OF THE BRAZILIAN POPULATION STRUCTURE AND ITS ANCESTRY BASED IN Y-STR HAPLOTYPES REGISTERED IN THE YHRD DATABASE

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Introduction: Haplotypic data integration of Y-chromosome Short Tandem Repeat (Y-STR) from different Brazilian regions is only valid in the absence of population substructure. Objectives: To evaluate the Brazilian population structure based in haplotypic Y-STR data in Y Chromosome Haplotype Reference Database (YHRD-53rd update), as well the male lineage ancestry in country. Materials and Methods: Pairwise genetic distances were carried out based on RST with the AMOVA method in Brazilian Admixed, Native-American and European populations typed for YFiler® system, involving 6,147 haplotypes. To the ancestry evaluation were inferred haplogroups based in Y-STR haplotypes with the Haplogroup Predictor software. Results: After Bonferroni correction, significant P values have been found in 128 among 153 pairs of Native-American populations ($P \leq 0.0003$; $0.0391 \leq RST \leq 0.9933$) and seven among 435 pairs of Admixed populations ($P \leq 0.0001$; $-0.0097 \leq RST \leq 0.0536$). Just one pair of Admixed populations had $RST > 0.05$ (Palmas-Santa Catarina

Valley, RST=0.0536), but with insignificant P value. Between European populations, just one pair (São Paulo-Porto Alegre) among three showed significant P value (P≤0.0167; RST=0.0307). Analysis involving 3,407 haplogroups of Admixed populations revealed an essentially European ancestry in Brazil (82.9%), highlighting the South (88.4%) and Midwest (85.0%) regions. Conclusion: Considering the YFiler® system, the Admixed Brazilian Metapopulation doesn't show population substructure, however, the substructure is evident in the Native-American Metapopulation of the country, so a unified database can't be used for this last one. About the Brazilian European Metapopulation, the few data recorded in YHRD suggest low genetic differentiation. The Brazilian Admixed populations revealed an essentially European ancestry, with little African and Native-American influence.

97. ANCESTRY AND STRUCTURE OF AFRICAN-BRAZILIAN POPULATIONS ESTIMATED FROM 46 AIM-INDELS

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46 AIM-Indels [1] is a panel developed to detect population structure and ancestry. Here, we add to the hall of worldwide populations genotyped for that set, which we chose for its high power in differentiating the main parental populations to Brazil. Brazilian populations were formed by an intense, complex, and heterogeneous process of admixture encompassing mainly Amerindians, Sub-Saharan Africans, and Europeans. Quilombos are Brazilian populations of marked African descent that have remained genetically isolated to some extent from surrounding populations. We present 46 AIM-Indels' allelic frequencies in three Quilombos (Kalunga, Mocambo, and Sacutiaba), and describe the populations' structure and ancestry. We have included data on African, European and Amerindian populations [2] in our comparative analyses and ancestry estimates. Descriptive parameters were estimated using ARLEQUIN v3.5 [3]. Population structure was assessed by PCA (R v3.2) [4] and STRUCTURE v2.3 [5]. Ancestry was estimated by STRUCTURE v2.3. Allelic distribution was intermediate to those found on the parental populations. Structure analyses and PCA showed the Quilombos are not affiliated to any specific parental, but are composites. That distribution is also reflected on admixture estimates: a higher African contribution, followed by the European and Amerindian contributions, in that order. All our estimates agree with the three Quilombos history and demographic dynamics, allowing us to assert that this AIM-Indels panel is a perfectly adequate system for studying our admixed populations.

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98. ANCESTRY CHARACTERIZATION OF HIGHLAND MESTIZO ECUADORIAN POPULATION USING AUTOSOMAL AIM-INDELS

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Ancestry Informative Insertion-Deletion markers have been used as a tool to infer the origin of population. In the present study, we report the results obtained for 46 AIM-INDELS of the highland Ecuadorian population by analyzing 171 unrelated individuals that have signed the informed consent for genetic studies. Samples were processed following the recommendations from Pereira et al. (2012). Lastly, the results were compared to reference population (Europeans, Africans and Native Americans), Native American was the most prevalent ancestry in highland Ecuadorian population with 63.1%, followed by European ancestry with 30.3% and lastly African ancestry with 6.6%. That admixture found in the present study is in agreement with the historical background of Ecuador.

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99. ANCESTRY ESTIMATES IN AFRODESCENDANT POPULATION FROM SAN BASILIO DE PALENQUE, COLOMBIA

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San Basilio de Palenque is located in Colombia, and was founded by escaped slaves mainly from Cartagena in the sixteenth century. For its history, traditions, ethnicities, culture and language, San Basilio de Palenque has been declared as Intangible Cultural Heritage of Humanity by UNESCO. Insertion/deletion polymorphisms (InDels) have considerable potential in the field of identification and ancestry assessment, since they combine desirable characteristics as genetic markers as simplicity of

analysis through PCR and capillary electrophoresis. In the present work a sample from San Basilio de Palenque population (n=187) was characterized using 46 autosomalAIM-indels. A database was built for genotypic and allelic frequencies, and used to assess the genetic ancestry of this peculiar group. As expected for an Afrodescendant population, the most representative ancestry estimate was African with 81.2%, followed by 10.6% European, and 8.2% Native American contributions. The results obtained were compared with previous estimates for a sub region of the Department of Bolivar, called Montes de Maria, which also include San Basilio de Palenque. In the pre-Hispanic era, this region was populated by Zenú indigenous, who mixed with the Spanish settlers. The African ancestry in San Basilio is four times higher than in Bolivar. Interestingly, the second component in San Basilio is European while in Bolivar it is Native American. Our findings can be useful to investigate the genetic structure of Bolivar department and show the importance of considering the heterogeneity that exists when selecting reference populations for forensic applications.

100. ANCESTRY EVALUATION OF AN AFRO-DESCENDANT POPULATION SAMPLE OF THE DEPARTMENT OF CHOCÓ - COLOMBIA

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The Afro-descendant population represents 10.6% of the inhabitants of Colombia (42 million) and 82% of the residents of the Department of Chocó (505 thousand) (DANE general census 2005; http://www.dane.gov.co). The present population status of the Department of Chocó is the reflection of its historical past, initially inhabited by several Native American communities and later by both Europeans and Africans who arrived during the colonization process in the American continent. This work determined the ancestral component of these three ethnic groups in a population sample of 120 Afro-Colombian individuals born in the Department of Chocó. The ancestral components were calculated according to the methodology described in [1]. Arlequin software v3.5.2.2 was used to estimate allele frequency distributions, and to perform Hardy–Weinberg equilibrium analyses. The software STRUCTURE v2.3.4 was used to estimate the African, European and Native American admixture proportions in the Afro-Colombian samples. 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 MD406 marker was the only one that showed significant deviation for the Hardy-Weinberg test. The ancestry analysis for this population revealed a main African contribution (76%) with a minor contribution of European and Native American ancestries, 17% and 7%, respectively. These findings are correlated with the historical records of this Pacific region.

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101. ANCESTRY INFORMATIVE MARKERS FOR ASIAN

SUBCONTINENT

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Ethics determination of bodies is a critical part of forensic identification particularly in missing person identification or disaster victim identification. Several set of SNPs were introduced to forensic communities as a universal ancestry informative markers (AIMs) panel based on available population databases. For deep study in subcontinent, the desired SNP panel should be figured within regional reference populations. This study had figured the best set of AIMs for Thai population from three accessible databases, international HapMap, 1000 Genomes and ALFRED. There were 374 Thai unrelated samples included in the study with east, south and southeast Asia data (258 samples) for references. We introduce the efficient AIM panel to determine Thai ethnicity. The achievement to reduce a size of 24 AIMs panel was clearly stratified Thais out of other east Asia populations by principal component analysis (PCA). The major and minor allele frequencies of entire AIMs were compared between Asia countries and major continents.

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102. ANCESTRY STUDY IN ECUADORIAN POPULATION WITH MULTIPLE MYELOMA

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Multiple Myeloma (MM) is a type of hematologic cancer that has been described in afrodescendent population1. The alteration of plasmatic cells can generate Monoclonal gammopathy of undetermined significance (MGUS) before develop MM2. Ecuadorian population is a complex mixture between Native American, European and African. We present the ancestry study with the hypothesis if the Ecuadorian population with MM have more percentage of African ancestry?. Samples from bone marrow and blood were taken from 61 Ecuadorian patients and DNA was extracted from the conventional way and 46 AIM-INDELs were analyzed. Both type of samples showed the same results. Ecuadorian population with MM displayed a mixture between Native American, European and African. There was not significant difference between MM samples (0.0411, SD±0.696) and the controls (0.0363, SD±0.696) for African ancestry ($P = 0.652$), for Europeans ($P < 0.001$) there was a significance difference between MM samples (0.4346, SD±0.290) and the controls (0.6168, SD±0.306) and lastly the Native American ($P < 0.001$) ancestry with significance (MM: 0.5243, SD±0.290 and control: 0.3470, SD±0.299). No MM cases had MGUS antecedents and its incidence is low in Ecuador3 that could be associated to the mestizo ethnic group, similar to Mexico4. For the first time here we present the genetic ancestry of mestizo individuals with MM where the African ancestry probably did not influence in cancer origin.

Cases with a higher percentage of Native American ancestry probably have more risk to develop MM. The mestizo ethnic group has its own behavior which explains the differences in clinical data in relation to other populations5.

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103. ANCESTRY PREDICTION IN QATARI POPULATIONS USING MISEQ FGX

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SNPs change approximately once every 10-8 generations while STR mutation rates are approximately one in a thousand. Because of this much lower mutation rate, SNPs are more likely to become "fixed" in a population than are STRs. SNPs are thus usually the better predictors of ethnicity (1). 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 Qatari populations. To develop ancestry prediction capabilities for population in this middle east region. 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. These data serve as the first Middle Eastern population was used as a testing data set to benchmark the ability of the selected SNPs to predict biogeographic ancestry using Miseq FGx. The results of this testing are presented herein.

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104. AUTOSOMIC STR DATABASE FOR AN

AFRODESCENDANT POPULATION SAMPLE OF SAN BASILIO DE PALENQUE, COLOMBIA

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The STR are a widely used tool in forensic genetics and biological anthropology. This study established allele frequencies and some parameters of forensic interest for the autosomal markers included in the PowerPlex® 16 BIO System kit (Promega). The sample population is from 151 unrelated afro descendant individuals of San Basilio de Palenque, Colombia, a population that is the last settlement that remembers the arrival of the Africans to America and that lacks databases published for these markers. DNA was isolated by salting-out. The amplified products with the PowerPlex® 16 BIO System kit (Promega) were separated by electrophoresis on 6% polyacrylamide gels and were visualized with an FMBIO IIe Genetic Analyzer (HITACHI). Allelic diversity and Hardy-Weinberg disequilibrium were performed using the ARLEQUIN software. Bonferroni correction assumes 0.05 significance level used for 15 tests yields an actual significance of 0.0033. The forensic parameters were calculated with PowerStats software (Promega CO). All markers analyzed showed heterozygosity between 66,89% (TH01) until 87,42% (Penta E and Penta D). All systems were in Hardy-Weinberg equilibrium (P <0.0033) after Bonferroni correction. The probabilities of paternity (W), exclusion (PE) and discrimination (PD) accumulated for loci analyzed were 0.99999899, 0.999999 and > 0.99999999, respectively. The parameters of forensic interest had values suitable for routine use in forensic genetics.

105. CATALOGING THE HIGHLY POLYMORPHIC STR REGIONS USING HIGH COVERAGE WHOLE GENOME SEQUENCING DATA FROM 1,070 JAPANESE INDIVIDUALS

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Short Tandem Repeats (STR) typing is widely used in forensic profiling. In cases that paternity testing or sibship testing for identification, or a close relatives become a problem in criminal case judgments, it becomes possible to examine with higher precision by using more polymorphic genetic loci. Thus, the highly polymorphic loci are demanded.
Related to this, we have cataloged the STR loci especially highly polymorphic in Japanese. The genetic diversity of the STR loci was comprehensively analyzed using the genomic data of 1,070 Japanese individuals[1)] obtained by the whole genome sequencing (WGS) based on the heterozygosity as the index. 1,638,516 STR loci repeated one to six bases found in the reference sequence of the human genome hg19 were analyzed using available STR analysis software.
Based on this result, we selected the STR loci that were estimated to be suitable for the appraisal STR typing with the following three criteria, 1) the locus with five or more variations of allele, 2) the locus with call rate exceed 0.95, 3) both expected heterozygosity and observed heterozygosity exceed 0.8. On autosomes, there are 388 loci passed these conditions. The length of these 388 STR loci are within 162bp, the length of short read of WGS. Thus, we could select the STR loci which are applicable to

actual forensic STR typing.
We are investigating these candidate loci for the appraisal typing using other STR typing experiments.

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106. CONTRASTING ADMIXTURE ESTIMATES IN RIO DE JANEIRO OBTAINED BY DIFFERENT SAMPLING STRATEGIES

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Brazilian populations are highly admixed, with Native American, European and African contributions that vary along the country. In this study, 1042 samples of non-related individuals from Rio the Janeiro State were genotyped for 46 AIM-Indels. The results showed a prevalence of the European ancestry (50.07%), followed by an African contribution (31.42%), and Native American background was less represented (18.51%). This sample was divided in subsets including volunteers from research works (2014-2016) and selected from paternity casework (2002-2004 and 2010-2016). The average ancestry was the same for samples from volunteers and paternity casework in 2002-2004. However, lower European and higher African ancestries were observed for paternity groups from 2010 onwards. Native American ancestry presented low variation between the groups of volunteers and paternity, as well as along the evaluated period. Comparing the residence place of the individuals in each subsample, differences were observed in the representation of each municipality in Rio de Janeiro State, and higher proportions of African ancestry were observed in samples from outside Rio de Janeiro metropolitan region, which is the case of the paternity subsamples. In conclusion, our results show that the ancestry values obtained for the global samples did not represent well the profile of the reference samples from paternity casework and emphasizes the importance of sampling strategies in admixed populations. These differences should be considered when performing paternity investigations involving individuals coming from different regions of Rio de Janeiro State, by using regional databases or by applying a correction for substructure.

107. DIP-STR HAPLOTYPE MARKERS FOR ANCESTRY INFERENCE

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The use of ancestry informative markers to investigate the population of origin of an unidentified DNA sample can provide further information from evidentiary specimens. Although excellent results have been obtained with lineage markers, Indels and SNPs, and several validated marker-sets exist, the current geographic resolution can potentially be improved by combining different types of markers through haplotype analysis.
Here, we aim to evaluate the contribution to ancestry inference of DIP-STR markers. DIP-STR

haplotypes offer the advantage of combining low mutation rate Indels (DIPs) able to assign individuals to continents; with high mutation rate STRs informative of populations of origin (1-2).
We assessed the ability of an initial set of 24 DIP-STRs to cluster the HGDP-CEPH reference populations, applying the STRUCTURE Bayesian clustering algorithm. The results at K=5 clusters, after excluding geographically close populations (Middle East and Central South Asia), show a clear pattern of five clusters corresponding to the major geographic regions of Africa, Europe, East Asia, Oceania and Native America. The results at K=7 analysing the complete HGDP-CEPH dataset, show that Middle East and Central South Asia form less distinct clusters and Europe loses clear definition from partial membership with additional inferred clusters.
Individual ancestry assignment using the Snipper likelihood-based system shows classification success rates higher than 99% in the five group analysis, while, in the seven group analysis, samples with Eurasian origin were more difficult to classify, with about 4% of Europeans, 13% of Middle Eastern individuals and 18% of Central South Asians miss-classified to neighboring Eurasian populations.

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108. DNA DATABASES OF HETEROGENEOUS POPULATION: POPULATION-BASED OR SUBPOPULATION BASED? A CASE STUDY OF THE LARGEST URBAN AGGLOMERATION OF PAKISTAN

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Construction of population database across a heterogeneous population must represent each subpopulation proportionately. It is hypothesized that disproportionate representation of subpopulations may lead to invalid outcomes. Karachi the largest urban agglomeration of Pakistan was chosen as the study area. One hundred and seventy five individuals from five subpopulations Baloch, Muhajir, Pathan, Punjabi and Sindhi were genotyped for three Short Tandem Repeat (STR) loci TPOX, CSF1PO and TH01. STR database was categorized as: (i) *Target database:* It included genotype profiles of the individuals from one of the five subpopulations. (ii) *Cognate database:* It included genotype profiles of all the individuals including individuals of the target database. (iii) *Noncognate database:* It included genotype profiles of all the individuals excluding individuals of the target database. In cognate and noncognate database, subpopulations of the individuals were not considered. Likelihood of each individual of target database to cognate and noncognate databases was calculated using GeneClass2. Therefore cognate and noncognate were used as *reference* databases. Results showed that the likelihood of each individual of target database to cognate database was higher than noncognate database. Higher likelihood to cognate database was consistent across all the five subpopulations. Hence it can be inferred that if a particular subpopulation is underrepresented in a database the likelihood of the individuals from that subpopulation to belong to the reference database was markedly decreased. Therefore, disproportionate population database highly undermine the potential of STR databases for medico-legal caseworks.

109. DNA PROFILING OF THE BULGARIAN TURKS FOR

FORENSIC IDENTIFICATION

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When forensic DNA analysis is performed, if there is a matching of DNA profiles as a result of the comparison, there is always a need for a useful data which will reveal the strength of the relevant evidence and make this match meaningful. One of this important data used for forensic genetics is the frequency of indistinguishable matching DNA profiles in the population. Allelic frequencies of STR loci vary from population to population. In the statistical computing performed for the evaluation of the DNA analysis results, it is important which population database will be used.
Our country, which is located on major migration routes and hosted many civilizations, has many races and ethnic groups. This study aimed to determine the genetic frequencies of the 24 STR loci which were determined by CODIS for the Bulgarian Turks who were born and lived in Bulgaria and settled in Turkey and use these results in the forensic laboratories in our country. In this study, intravenous swab or blood samples of 150 people whose age ranged from 18 to 90 years and who have given informed consent were used.
DNAs were isolated from the collected samples using QIAAMP DNA Mini Isolation Kit. The DNA concentrations were measured and then PCR process was performed with GlobalFiler STR Kit. After this PCR procedure, PCR products were run on ABI 3130 Genetic Analyzer to view the amplified DNA sequences. The results of our study will be presented at the congress.

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110. EVALUATION OF ANCESTRAL MEMBERSHIP PROPORTIONS AND GENOTYPE DISTRIBUTION IN THE PERCEPTION OF UMAMI TASTE IN ECUADORIAN MESTIZOS

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The study of genetic factors on taste sensitivity characterize different populations on the perception of flavors and consequently, food preferences, dietary habits, and health risks. The umami taste is found in monosodium glutamate (MSG). The T allele of SNP rs307377 of TAS1R3 gene influences the levels of perception to the MSG. The aim of this study was to evaluate the genotype distribution of the single nucleotide polymorphism involved in the perception of Umami taste, and the ancestral membership proportions in the mestizo Ecuadorian population. A total of 748 participants were analyzed. Descriptive statistical analysis was used and HWE was determined. 100% of the participants were found as

supertasters and the C/C genotype was fond in all samples. The ancestry of all participants was determined using 46 autosomal Ancestry Informative Markers (AIMs). A greater Native American ancestry proportion (0.51) was shown, compared with the European (0.45) or the African Native-American groups (0.048). These results allow us to characterize and differentiate Ecuadorian mestizo population. It is known that ethnic groups differed significantly from one another in reported perceived taste intensity studies. Hispanics taste sensations are higher than non-Hispanic Whites. Genetic influence within the perception of umami flavor in South American individuals, contributes to the growing evidence that human traits are associated with variants that are outside of the coding regions and can clearly influence the phenotype.

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111. EVALUATION OF THE INNOTYPER® 21 GENOTYPING KIT IN MULTI-ETHNIC POPULATIONS

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The forensic community has for long focused on the multiallelic loci of the class of Short Tandem Repeats (STRs). Recently there has been a shift of focus to shorter binary markers (InDels[1] and INNULs[2]) which have the advantage over STRs in being devoid of stutter and have smaller amplicon sizes (< 130 bp).
In this study we evaluated InnoTyper® 21 for use in South African populations by testing 507 individuals. We identified one novel off-ladder variant in the Bantu populations (frequency = 0.015) in locus RG148, this variant was characterized by Sanger sequencing the amplicons. As expected the highest combined random match probability (CMP) and combined discriminatory capacity (CDC) was identified in the admixed population 6.94x107 and 0.999999985 respectively. The lowest CMP and CDC was identified in the Bantu groups displaying values 8.09x106 and 0.99999987 respectively, similar to other studies [3–5]. All loci were in Hardy-Weinberg Equilibrium (HWE) following Bonferroni correction. Significant population differentiation (Fst) was observed between the African and non-African groups. To conclude the InnoTyper® 21 genotyping kit will make an admirable addition to the contemporary STR and mtDNA genotyping methods when dealing with degraded genetic material.

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112. FORENSIC PARAMETERS OF THREE STR MARKERS

(D22S1045, D2S441 AND D10S1248) IN THE POPULATION FROM SÃO PAULO STATE, BRAZIL

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In this work we analyzed statistical data of three markers STRs (D22S1045, D2S441 and D10S1248) that are used in our laboratory's forensic routine for paternity investigations. The use of these data will be extremely important for the laboratory since there are no allele frequencies data for such markers in the literature, which made it difficult for the laboratory to perform statistical analyzes for the conclusion of the reports. We analyzed 221 samples obtained from unrelated individuals born in the São Paulo state, which is considered to be the Brazilian state with the highest cultural and ethnic miscegenation in the country, mainly descendants of Italians and Portuguese, but also strongly influenced by Amerindians, Africans, Spaniards , Germans, Arabs and Japanese, as well as the high number of immigrants from the northeast region. The allele frequencies and statistical parameters were estimated with PowerStats version 12 (Promega Corp.).The power of discrimination (PD) and power of exclusion (PE) for the marker D10S1248 were 0.914 and 0,576 respectively and the allele with the highest frequency was 14 (0.294) and the lowest frequency were alleles 8, 10, 18 and 19 (0.002). The PD and PE of the marker D2S441 were 0.907 and 0,551 respectively and the allele with the highest frequency was 11 (0.312) and the lowest frequency were alleles 9 and 12.3 (0.002). The marker D22S1045 had PD 0.890 and PE 0,489 with a higher frequency of allele 16 (0.373) and less allele 8 (0.002).

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113. GENETIC CHARACTERIZATION OF THE BRAZILIAN IMMIGRANT POPULATION IN LISBON WITH INDEL GENETIC MARKERS

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Migration is one of the main factors for genetic variability within populations. Currently, the Portuguese population, and particularly the population from Lisbon, welcomes a considerable number of immigrants. Brazilian immigrants are the main foreign community in Portugal, with about 80 000 individuals in 2015.

Insertion/deletion polymorphisms - InDels -, are characterized by the presence or absence of small DNA sequences. These variations constitute a group of genetic markers with advantages for forensic identification, especially in highly degraded biological samples, due to the small size of the amplification fragments. Furthermore, InDels not only are useful for complex cases of biological kinship, but they also have lower mutation rates.
Presently, there is no available data for InDel markers of the Brazilian immigrant population in Lisbon. Thus, our aim is to characterize this population by typing a group of individuals with a panel of 30 InDel. Therefore, we studied 181 Brazilian immigrants. Samples were typed with the Investigator DIPplex® Kit. Fragments were detected by capillary electrophoresis using the ABI PRISM 3130 *xl* automated sequencer and results analyzed with GeneMapper v1.2 software. Statistical inference of data was carried out with Arlequim v3.5 software.
With Investigator Diplex Kit ® complete genetic profiles were obtained for all individuals. The achieved genetic data allowed us to evaluate genetic distances between Portuguese population and immigrant populations with available genetic data. Our results confirm genetic differences between Portuguese population and Brazilian and PALOP´s immigrant populations.

114. GENETIC DATA FOR TWENTY TWO AUTOSOMAL STRS (POWERPLEX FUSION) FROM AFRO-ECUADORIAN POPULATION

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The PowerPlex Fusion System is a multiplex that includes twenty four loci, between them twenty two are autosomal (D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433, FGA and D22S1045). According to an Ecuadorian census (2010), Ecuador has 1.042.812 Afro decedent population (7.2% of the population). Genetic data for the 22 STRs was obtained from non-related samples from Afro-Ecuadorians who has signed the informed consent for population genetic studies. The samples were amplified in a multiplex following the protocol recommended by the manufacturer. Moreover, the fragments separation and detection was done in 3500 Genetic Analyzer (Applied Biosystems), results were collected with Data Collection v4.0 and evaluated by Gene Mapper v5 (Applied Biosystems). Allele frequencies and other forensic statistical parameters were estimated. Due to the fact that there is not enough genetic information about Afro individuals from Ecuador, here we present complete data.

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115. GENETIC DIVERSITY OF 23 AUTOSOMAL STR LOCI IN A TIBETAN POPULATION

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The genetic polymorphisms of 23 autosomal short tandem repeat (STR) loci included in the Huaxia Platinum System were evaluated in 200 unrelated healthy individuals of Chinese Tibetan population living in the Tibet Autonomous Region. All of the loci reached the Hardy–Weinberg equilibrium. Totally 233 alleles were observed with corresponding allelic frequencies ranging from 0.0025 to 0.5900 and forensic statistical parameters was calculated. The total discrimination power and combined probability of exclusion of the 23 STR loci were over 0.99999999999999999999999999 and 0.999999999, respectively. Results suggested that the 23 STR loci had a relatively high genetic variation, which was suitable for forensic personal identification and paternity testing in Tibetan population.

116. GENETIC DIVERSITY OF A MEXICAN-MESTIZO POPULATION USING 114 INDELS POLYMORPHISMS

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Insertion–deletion polymorphisms (indels) are binary markers with considerable potential in the forensic field, since this variation can be analyzed in short amplicons, improving successful amplification of highly degraded samples; in addition to its low mutation frequency that makes them suitable for kinship testing. Moreover, indel polymorphisms with large differences in allele frequencies between ethnicities can be useful to infer the biogeographic ancestry of the donors of forensic evidence. For this study, we genotyped a set of Mexican population sample (n=154) using 114 indel markers, 68 of them have been proposed for forensic use (Investigator DIPplex and 38 HID-Indels) and 46 constitute Ancestry-informative markers (AIM-INDELS) (Pereira-et-al. 2012). The sample included admixed populations from different geographic regions: northern (Sinaloa), West (Jalisco), Center (Mexico City), and Southeast (Yucatan). Allele frequencies and genetic diversity were calculated for all 114 indel loci. The Hardy-Weinberg equilibrium was demonstrated for 113 loci, only the 360-marker (AIM-INDEL) showed significant value; and linkage equilibrium test showed only departure for the HLD45/HLD64 combination in the 68 indel loci. Additionally, discrimination power (DP), random match probability (RMP), power of exclusion (PE), typical paternity index (TPI), and polymorphic information content (PIC) were calculated for the 68 indels used in human identification. The combined power of discrimination and exclusion was greater than 0.999999999999999 and 0.999996089797308, respectively; and the random match probability value was less than 5.544 x 10-22. The ancestry proportions were also assessed using the 46 AIM-INDELS, getting differences between regions, mainly for the Native American (0.433-0.692) and European (0.522-0.257) components.

117. GENETIC POLYMORPHISMS OF 20 INDEL MARKERSIN CHINESE HEBEI HAN POPULATION

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Insertion–deletionpolymorphisms(InDels)for human identification purposeare basicallylength polymorphisms caused by insertion or deletion of 2~6 bp approximately,which arewidely distributed throughout the genome. There are several advantages of Indel for forensic application, such as relatively short amplified fragments, lower mutation rate, and without stutter products detected using capillary electrophoresis, whichmake it possible to analyze highly degraded DNA samples and complex paternity cases.In this study, a set of HeBei Han population samples (n=206) were genotyped using InnoTyper™ 21 kit (Inno Genomics, USA) which contains 20 InDel markers and amelogenin.Allele frequency, observed heterozygsity(Ho), expected heterozygsity(He), polymorphism information content (PIC), probability of match (PM), power of discrimination (PD), typical paternity index (TPI) and power of exclusion (PE) were calculated for these loci. There was no significant deviation of Hardy-Weinberg equilibrium from expected values (*P*>0.0025,after Bonferroni correction for multiple testing).Combining of the 20InDel markers, the random probability of match was 2.7615×10^{-7}, the PD and PEwere0.9999997238 and 0.9114432617, respectively.On the whole,the application of Indel genotyping as a supplementary tool for degraded DNA and challenging kinship studies would improve human identification accuracy.

118. GENOME-WIDE ANALYSIS OF PRESENT DAY ARGENTINEAN MENNONITES

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The first settlers of the Argentinean Mennonite Colony 'La Nueva Esperanza' arrived in 1985. The founding members of this community originated from the Mexican and Bolivian ones, and belong to the Anabaptist branch known as 'The Community of Mennonites from the Old Colony of Reinland'1,2. The 2005 internal census yielded a population of 1278 individuals. Historical records and Y-chromosome studies indicate that pioneers of this colony came from Central Europe, pointing to the Netherlands as the core of the original Mennonite diaspora. Aiming at studying this Mennonite Argentinean population from a genome-wide angle we analyzed a panel of more than 580.000 autosomal SNPs in individuals living in 'La Nueva Esperanza'. Different SNPs genome repositories from diverse human populations were intersected with the SNP data obtained from the Mennonites. The 1000 Genomes Project data provided the dataset with the largest SNP overlap with the Mennonites. The results obtained in the present study are in good agreement with those obtained in the analysis of Y-chromosome for the same Argentinean Mennonites, particularly in that the ancestry is virtually 100% European and in the

presence of a probable greater level of endogamy in Mennonites compared to other Argentinean and European populations. The variation observed in Mennonites is coherent with their known style of life and mating rules, and could also explain a higher incidence of certain Mendelian diseases in these small communities3-4. The present study contributes to understand patterns of genetic variation in populations from Argentina, which are fundamental in forensic genetics.

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119. INVESTIGATION OF BRAZILIAN POPULATION´S GENETIC STRUCTURE THROUGH A SNPS IDENTITY MARKERS PANEL: FREQUENCIES DETERMINATION, FORENSIC PARAMETERS, SUBSTRUCTURE OCCURRENCE, DATABASE STRUCTURE REPERCUSSION AND Y-HAPLOTYPES ANALYSIS ON A MASSIVE PARALEL SEQUENCING PLATFORM

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Current criminal forensic practice in Brazilian Forensic Genetic facilities rely mainly on STRs analysis assessed through capillary electrophoresis. Previous studies found no evidence of substructure occurrence among the country´s different regions, based solely on STR data. No laboratories presently features technical resources to implement alternative markers use, especially due to lack of information on such markers distribution in the Brazilian population. The present work was designed to characterize the Brazilian population regarding a 124 SNPs markers panel, including 90 autosomal and 34 upper Y-clade markers (HID-Ion Ampliseq Identity Panel, ThermoFisher). 400 human male samples (comprising 80 samples from all five Brazilian geographic regions) were genotyped using a Ion-PGM Sequencer (ThermoFisher) on six 318 chips. Data were analyzed with a set of pertinent plug-ins, scripts and softwares. Obtained frequencies, forensic parameters and other relevant information suggest this technique has a potential use in forensic cases involving Brazilian samples, specially where samples have a high degradation level (smaller amplicons are generated in this method) or high throughput is necessary (DVI cases with a large number of victims, for example). Ethnicity-based genetic structuration of the Brazilian population, assessed by the Y chromosome haplotype, was investigated and compared to the current model, where total admixture is assumed. Implications of the findings also impact the ongoing database construction models. Finally, an analysis of the Y chromosome haplotypes is presented, consonant with accepted admixture models in Brazil, where European origin´s Y haplotypes have, due to historical and social reasons, highest prevalence.

120. MOLECULAR ANALYSIS OF ANCESTRY INFORMATIVE MARKERS (AIMS-INDELS) IN A HIGH ALTITUDE ECUADORIAN WOMEN POPULATION AFFECTED WITH BREAST CANCER

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Breast cancer (BC) is the leading cause of cancer-related death among women in 2014. BC is caused by genetic and epigenetic aberrations. BC is a heterogeneous group of tumors with molecular characteristics according to subtyping differing in genomic complexity, key genetic alterations, and clinical prognosis. Given the description of the Ecuadorian population as multiethnic and multicultural, made up of African, Amerindian natives, and European groups1, this research is an initial evaluation of the potential benefits that would be obtained by generating a haplotype map of the Ecuadorian population to design research and therapy associated with population genetics2. The goal is to estimate the original proportion of each inferred population and to determine the underlying population in women affected with BC3. The ancestral proportion among African, European, and Amerindian natives in Ecuadorian women was calculated through 45 ancestry informative markers (AIMs) and the comparison to the Human Genome Diversity Project panel4, 5. The resulting allele frequencies in Ecuadorian women affected by breast cancer indicate prevalence of the Amerindian native ancestral component with 60.58%, and minor proportion for the European and African components with 34.57% and 3.7%, respectively. These results suggest that the genetic variations expressing breast cancer in Ecuadorian women could have been caused by the insertion of certain genetic characteristics of the American native groups as consequence of ancestral migration towards South America.

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121. NOVEL IDENTIFICATION OF RARE AND VERY SMALL OFF-LADDER ALLELE AT TPOX LOCUS OF AUTOSOMAL STR

MARKERS

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Short tandem repeat (STR) loci in human genome have been widely used for human identification in forensic casework1. In this study, we discovered rare and very small off-ladder alleles at TPOX locus among several autosomal STR markers in sexual assault casework. So we tested samples originated from blood, pubic hair and nail of female victim using by the most commercial multiplex PCR systems; AmpFlSTR® Identifiler®, PowerPlex®16 HS System and PowerPlex® Fusion Systems, showing results that 1-repeat and 8-repeat due to heterozygote. The results obtained from 3 multiplex PCR system were identical each other. Therefore, we partially amplified only STR region of TPOX from the female genome and cloned into TA-cloning vector, and then sequenced for the revaluation of STR typing results. In result, it demonstrate that detection of 'AATG', one time repeat sequence in STR region of TPOX was consistent with former STR typing results. The TPOX locus has at least 13 different alleles ranging in size from 4 to 16 tetranucleotide repeats2, but, in this study, we detected the existence of very small allele at the TPOX locus that has been not yet reported. Consequently, it may cause misreading of STR at other locus beside TPOX locus in multiplex PCR systems. Taken together, we suggest that unusual alleles, 1-repeat in TPOX locus in Korean population could be detected very rarely. Therefore, when analyzing alleles of TPOX locus from the samples in forensic casework, it may have to be careful in overleaping very small off-ladder alleles.

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122. OPTIMIZATION AND VALIDATION OF MISEQ FGX FORENSIC SIGNATURE KIT IN KOREAN POPULATION

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The most utilized method of DNA profiling is the Capillary Electrophoresis (CE). But nowadays, NGS technique also has become of great interest to those researching forensic genetics. NGS kits for DNA profiling are also being continuously launched which include makers for autosomal, X & Y chromosomal, mitochondrial DNA as well as Single Nucleotide Polymorphisms (SNPs), and these not only provide genetic profiling information but also racial and phenotypic estimation results.
This study aims to analyze 59 STR markers of 300 Korean samples and to optimize and validate the MiSeq ForenSeq Sequencing Kit (Illumina). During the optimization process we obtained reliable data from 288 samples. All data set was put in a table by the recommendations for NGS data nomenclature of ISFG. Moreover, a concordance test was carried between Illumina's ForenSeq Sequencing Kit and CE based STR profiling kits (GlobalFiler PCR Amplification Kit, PowerPlex Y23 System, etc.). As part of validation, the reliability of output data of the average depth of coverage (STR loci, allele, and stutter) was checked. Most of markers were excellent but there were unbalanced markers such as DYS385, DYS612, and DYS387S1.
In this study, we examined the application of the commercialized NGS kit for human identification and

analyzed detailed frequency in the Korean population. Results from this study will be helpful reference points and have a statistical significance to other research group that use NGS.

123. PARAGUAY: UNVEILING MIGRATION PATTERNS WITH ANCESTRY GENETIC MARKERS

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Before the arrival of European settlers to Paraguay, its Eastern region, was inhabited by Guarani people. The inexistence of mineral wealth quickly decreased the interest of Spanish colonizers in this territory, turning Paraguay into an isolated region, geographically, culturally and politically separated from the other economic forces from the colonial period in south America. After the Paraguayan war in 1870 that ended in a loss of 60%-70% of the male population, the immigration to the country was encouraged. Large flows of Brazilians and Argentineans arrived since the 1960s. Immigration data also indicate high input of Eurasians (Germans, Koreans and Japanese). Samples from the eastern provinces of Paraguay (n=300) were sequenced for the mtDNA control region and 88% presented native American haplogroups. A preliminary study on the same samples using AIMs indicates a high autosomal contribution from Europe (around 60%) and native America (around 30%). The comparison of both type of markers shows that the European ancestry for autosomes is higher than expected when averaging mtDNA and the Y chromosome. This result supports recent admixture between Paraguayans and other populations probably already admixed, where the men contributed with high European ancestry. Moreover, it highlights the demographic importance of the admixture events that succeeded the first interaction between European males and native American females during colonial times, and the role of the following gene flow in modulating the genetic composition of the current Paraguayan population.

124. POLYMORPHISM OF 18 SHORT TANDEM REPEAT LOCI OF HUI POPULATION FROM GANSU REGION

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ABSTRACT: Objective The aim of this study was to determine the frequencies and parameters of Hui population 18 short tandem repeat loci (D5S818, D8S1179, D7S820, CSF1PO, D2S1338, D3S1358, vWA, D21S11, D16S539, PentaE, TPOX, TH01, D19S433, D18S51, FGA, D6S1043, D13S317, D12S391) in Gansu region. Methods PCR amplification and capillary electrophoresis technologies were

employed to determine the genetypes of 18 STR loci for 1038 unrelated Hui individuals. Results 223 alleles and 982 genotypes were recognized in Hui population from Gansu region. The results demonstrated that all loci were found to be no deviation from Hardy-Weinberg equilibrium(P > 0.05). Heterozygosity(H) ranged from 0.601 to 0.929, matching probability(Pm) ranged from 0.012 to 0.213, power of discrimination(DP) ranged from 0.787 to 0.988, polymorphism information content(PIC) ranged from 0.550 to 0.920, power of exclusion(PE) ranged from 0.292 to 0.854. Conclusion This work studied the genetic polymorphism of 18 short tanderm repeat loci of Hui population in Gansu region. The resulting genetic data to provide basic data for subsequent human population genetics and forensic DNA research work.
KEY WORDS: Population genetics; DNATyperTM19; STR Polymorphism; Gansu Hui population

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125. POPULATION DATA OF NEW 21 MINI-INDELS FROM TURKEY

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Insertions/deletions (InDels) are a variety of polymorphisms that are observed with deletion or insertion or both and common on both somatic and gonosomal chromosomes. Since last decade InDels has been implemented to forensic field as an alternative marker for forensic identification. In forensic sciences, sex chromosomes becomes essential for the specific cases. Thus the relationship between father and daughter can be determined by using of X chromosome polymorphism such as X- InDels. In this study we aim to determine population genetic data of 21 gonosomal mini InDel loci (including 20 X-chromosomal and 1 Y-chromosomal) in Turkish population (1). We collected 150 blood or buccal swab samples from non-relative healthy volunteers. PCR and electrophoresis conditions were applied according to the protocol recommended by (2). Forensic and population genetic parameters of the 21 InDels were estimated with Promega PowerStats excelsheet, p-values of exact tests for Hardy–Weinberg Equilibrium and the test of population differentiation were calculated with Arlequin ver. 3.5.2.2. The mean heterozygosity ratio of 21 loci was determined as 0.400 and the discrimination power was defined as 0.99. The result of this study show that gonosomal 21 InDel loci are polymorphic for the Turkish population and required the discrimination power can be reached. Therefore, these InDel loci can be used separately or together with currently available STR and SNP loci for forensic cases.

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126. POPULATION GENETIC DATA OF 18 STR LOCI IN A KURDISH IRAQI POPULATION

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The accurate forensic kinship analysis of STR-based genetic data involves the use of allele frequencies from appropriate reference populations. We have received a large number of immigration cases from Iraq during the last decade and were lacking a proper set of Iraqi allele frequency estimations. Therefore, we have compiled genotype data from approx. 400 parentage cases to establish allele frequencies for 18 autosomal STRs in a Kurdish Iraqi population. A total of 780 individuals were selected based on their known birthplace in the Kurdistan Region in Northern Iraq. Genotypes resulted from STR typing with a combination of PowerPlex® 16 or 21 System (Promega) and either AmpFlSTR® SEfiler™ (Thermo Fisher Scientific) or Investigator™ Decaplex SE (Qiagen) Kit. The following STR markers were included: CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, Penta D, Penta E, SE33, TH01, TPOX and VWA. Allele frequencies, Hardy-Weinberg equilibrium, linkage disequilibrium, typical maternity and paternity indices as well as match probabilities and FST pairwise genetic distances were calculated with Arlequin v3.5.2.2 and compared to European1 and Southern Iraq2 allele frequencies.

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127. POPULATION GENETIC DATA OF 21 STR MARKERS IN THAIS OF SOUTHERN BORDER PROVINCES OF THAILAND

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Allele frequency and statistical parameters analysis of 21 STR loci were determined in 249 unrelated individuals from Thais of Southern Border provinces. 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. The Hardy-Weinberg Equilibrium and statistical parameters of forensic were examined including observed heterozygosity (Hobs), expected heterozygosity (Hexp), probability of exact test (P value), power of discrimination (PD), power of exclusion (PE), match probability (pM), polymorphism information content (PIC) and paternity index (PI). The study reveals that the expectations of the Hardy-Weinberg Equilibrium (HWE) were confirmed for all loci. SE33 is the most informative locus in this population. The combined power of discrimination gave an expected value of 0.99999999999999999999999999967 and the combined power of exclusion gave an expected value of 0.9999999987 for all loci. Allele frequencies and statistical parameters analysis value of 21 STR loci indicated the utility and efficacy that can be used for individual identification and paternity testing.

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128. SCREENING OF MULTI-ALLELIC INDEL MARKERS WITH MONOMERIC BASE PAIR EXPANSIONS FOR FORENSIC APPLICATION

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Insertion/deletion polymorphisms (Indels), with the characteristics of low mutation and short amplicons, have been regarded as promising markers for forensic DNA analysis comparing with traditional STR markers. Many researches contributed to the exploration of the di-allelic indels for forensic application. Multi-allelic indels, with approximately 20000 distributing in the introns of genome in1000 genome database, have received a little attention. In this study, seventeen multi-allelic indel markers, with around ten monomeric base pair expansions, were screened from 1000 genome database and combined in one multiplex PCR reaction system. A total of 100 unrelated Han individuals residing in Chengdu (China) were genotyped using our designed multiplex amplification system and capillary electrophoresis. Forensic genetic characters of the seventeen multi-allelic indels were analyzed. The combined discriminatory power (CDP) and the cumulative probability of exclusion (CPE) were higher than 0.999999, 0.9977, respectively. The results demonstrated that our indels multiplex panel was informative and polymorphic in the investigated population. The results also suggested that the multi-allelic indel markers with monomeric base pair expansions are useful for human identification and parentage testing.

129. TETRA-ALLELIC SNPS SCREENED OUT FROM 1000 GENOMES DATABASE AND FORENSIC GENETIC ASSESSMENT BASED ON CHINESE HAN POPULATION

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Up till now, single nucleotide polymorphisms (SNPs) have been widely used as a powerful genetic information source of forensic personal identification, ancestor information, complex relationship testing, and phenotypic inference. In the present study, to achieve higher forensic discrimination power, we established a multiplex SNaPshot minisequencing panel with multi-allelic SNPs. The data of CHB (Han Chinese in Beijing) and CHS (Southern Han Chinese) from 1000 Genomes Project database were analyzed using vcftools (http://www.internationalgenome.org/). A total of 48 tetra-allelic SNPs sites were

screened out as candidate SNP loci. After calculating the heterozygosity, Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), and evaluating the feasibility of primers design, only 7 tetra-allelic SNP loci can meet the requirement of forensic application and the multiplex construction. Thus, other 8 tri-allelic SNPs were selected to complement the final panel. The sizes of all amplicons were designed less than 190bp. A total of 120 unrelated individuals from Chinese Han population were genotyped. Forensic genetic assessment of the panel was performed with PowerStats software (Promega). The combined match probability (CMP), combined discrimination power (CDP) and cumulative probability of exclusion (CPE) were $6.24X10^{-10}$, 0.999999999376 and 0.997152, respectively. The results demonstrate that this panel is suitable for forensic personal identification and paternity testing.

130. THE VALIDATION ANALYSIS OF THE 27-PLEX SNP PANEL FOR ANCESTRY INFERENCE

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Previously, our lab set up a single-tube 27-plex Single Nucleotide Polymorphisms (SNPs) panel to infer ancestry components of unknown individuals in a three-continental level. Here we enhanced the accuracy of the system by establishing an analysis pipeline, including likelihood ratio, ancestry composition and individual ancestry assignment, based on a reference database of 3081 individuals from 33 global populations. The accuracy of the pipeline was evaluated by cross validation study using data from the database. The results show that the analysis pipeline was over 99% accurate when analyzing unknown individual's ancestry origin from East Asia, Europe, Africa and the admixture population. A test sample group contained 1096 individuals of 11 populations were analyzed using this pipeline. In total, 99.40%, 87.36%, 99.84% and 70.77% of East Asian, European, African and Admixture individuals were assigned consistently with their predefined geographical regions, respectively. While the inconclusive rate corresponding to the above mentioned regions are 0.60%, 12.64%, 0.16% and 29.23%, respectively. Most of the inconclusive result happened between Admixtures and European, or between Admixtures and East Asian, with a likelihood ratio within one order of magnitude, in this case, the ancestry composition provide a good indicator of ancestry origin of an individual.

131. TYPING OF TWO MIDDLE EASTERN POPULATIONS WITH THE PRECISION ID ANCESTRY PANEL: ANALYSIS OF THE RESULTS USING THE GENOGEOGRAPHER SOFTWARE

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Ancestry informative markers have a great potential for ancestry inference in forensic genetic casework. However, there is a high degree of uncertainty in determining the ancestry of an individual if that individual originates from a region that is poorly represented among the reference databases (DB). One such region is the Middle East. The aim of this study was to type two populations from the Middle East

using the Precision ID Ancestry Panel and to assess the panel's ability to differentiate geographically proximate populations.
Samples from 93 Iranians and 100 Turks were typed for the 165 ancestry informative markers. The genetic differentiation (FST) between Iranians and Turks was small (FST = 0.00209, p-value = 0.00396±0.0006). Principal Component Analysis (PCA) showed that Iranians and Turks clustered together indicating that the SNPs included in the Precision ID Ancestry Panel were not able to separate the two populations.
To apply ancestry informative markers in forensic genetics, it is necessary to calculate the weight of the evidence. The program GeneGeographer was developed for this purpose. GenoGeographer calculates a DB-score that indicates whether the individual belongs to at least one of the reference populations in the database. If this is the case, it calculates the population likelihood for each reference population and presents the confidence interval of each likelihood in a graphic interface that allows the user to interpret the significance of the ancestry inference. GenoGeographer will be used to evaluate the strength of the ancestry inference of the individuals from the Middle Eastern populations.
WC: 249

132. Y CHROMOSOME HAPLOGROUPING FOR ASIANS USING Y-SNP TARGET SEQUENCING

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Y-chromosomal haplogroups are sets of ancestrally related paternal lineages. We prepared a target sequencing panel consisted of 94 Y-SNPs to determine Y chromosome haplogrouping in Asian populations. The Y-SNP panel was applied to several Asian ethnic groups: Korean, Han Chinese, Japanese, Vietnamese, Pakistani, and Bangladesh. Y-SNP Target capture was done using the SureSelect XT Custom capture kit (Agilent) and NGS was performed using the HiSeq 2000 (Illumina). The average mean read depth of target sequencing was about 2,800X. The UCSC hg19 was used as reference sequence. Variant calling and annotation were performed by the GATK and ANNOVAR programs. In order to verify the accuracy of the target sequencing, we confirmed 99.7% accuracy rate of the selected markers by the Sanger sequencing method. We could provide a Y-haplogroup tree for Asian groups. We believe that the designed Y-SNP panel will be useful to determine the exact Y-haplogroups.

133. ANALYSIS OF NONBINARY SNP AND ADJACENT MICROHAPLOTYPE FOR FORENSIC PATERNITY TESTING

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Microhaplotypes and nonbinary SNPs have been proved to be effective forensic markers for human identification and paternity testing. In this study, we applied Next generation sequencing(NGS) with

pooling samples to select nonbinary SNPs and adjacent microhaplotypes. Five nonbinary SNPs and adjacent microhaplotypes (contained 3 binary SNPs) were chosen. Then, we established a detection system which employed single chain sequencing based Massively Parallel Sequencing Technology and PCR based Single Strand Conformational Polymorphism technology. The detection result revealed that the multiplex typing system had high polymorphism and it could be used to supply paternity testing.

134. ANALYSIS OF PATRILINEAL RELATIONSHIP IN RUSSIAN FEDERATION USING SEVERAL Y-STR MULTIPLEX PANELS

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For this study, we have analysed 287 cases of patrilineal relationship between individuals from various regions of the Russian Federation. Y-STR analysis was performed using Yfiler®, Yfiler® Plus (both Applied Biosystems, USA), and COrDYS-Y (GORDIZ Ltd, Russia) genotyping systems. The number of meioses between the individuals varied from 1 to 3 (based on self-declaration by the study participants). In 233 cases either a complete match of Y-STR profiles between alleged relatives (85.8%) or mismatches at no more than 3 loci (14.2%) were observed. In 54 cases, more than 3 mismatches between participants were detected and these samples where not taken for further analysis. When using Yfiler® (n=117) a complete match between participants was observed in 91.6% cases, 1 mismatch – in 7.6% cases, and 3 mismatches – in 0.8% cases. When using Yfiler® Plus (n=58) a complete match between participants was observed in 79.3% cases, 1 mismatch – in 20.7% cases, and 2 mismatches - in 3.4% cases. In cases analysed by COrDYS-Y (n=58) a complete match between participants was observed in 81.0% cases, 1 mismatch – in 13.8% cases, 2 mismatches - in 3.5% cases, and 3 mismatches - in 1.7% cases. All mismatches detected during the present study were by one STR repeat with a single exception of locus DYS627 (Yfiler® Plus) where a mismatch of two repeats was found in one case. Overall, mismatches were present at all loci studied with exception of DYS392, DYS437, DYS438, DYS448, DYS481 and DYS533. The highest mutation frequency was observed for locus DYS570 (0.0256).

135. IDENTIFICATION OF TWO RARE OFF-LADDER ALLELES OF D13S325

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We found two rare alleles of D13S325 from three paternity testing cases, which were located out of D13S325 locus and falsely labeled as allele of D21S1270 and D9S925 under the Microreader™ 23sp ID system (Microread, Suzhou, China). One allele was misread as allele 14.3 of D21S1270 in two cases and the other allele was misread as allele 15.3 of D9S925 in one case. These rare alleles were verified using the Goldeneye™ 22NC system (Peoplespot Inc., Beijing, China) and sequencing of D13S325 single locus amplification products. According to the sequencing results, these rare alleles were designated as allele 5.1 and 35.1 of D13S325 respectively. Because the rare allele 5.1 and 35.1 of D13S325 were incorrectly labeled in the Microreader™ 23sp ID and Goldeneye™ 22NC systems, particular attention should be paid when the systems are used in paternity testing and personal

identification. We suggest that it had better to redesign the primers of D13S325 to avoid the potential mistyping risk.

136. INTRODUCING TWO TYPES OF Y-STR APPLICATION MODELS IN CHINA

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The Y chromosome is male-specific and represents the paternal lineage. This feature can be used in database searches to locate a suspect through his relatives, but the traditional Chinese society is changing rapidly. On the one hand, China is a country with strong geographic ties, with big families settled in a fixed area, thus the population in a certain area is relatively stable. The 'Case-pedigree-individual' model was established to help limit the scope of an investigation and to determine which family the suspect belongs to in the enclosed area. On the other hand, with the continuous economic development and urbanization, more people swarm into cities to live and work, causing the collapse of the traditional rural society. This makes it possible that people in different cities come from the same town and are part of one big family. In this situation, 'Case-pedigree-birthplace-individual' model was applied to Y-STR investigations in open areas especially the big cities. We will introduce cases solved using both models in China.

137. MULTISTEP MICROSATELLITE MUTATION AT D18S51 LOCUS IN A PARENTAGE TESTING CASE

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Mutations of short tandem repeats (STR) loci might cause allelic mismatchs between the child and the parent, which entangled the forensic inference in paternity testing. Most of the reported microsatellite mutations are confined to single-step mutations, and multistep mutations were rarely reported and only account for a very limited number of STR mutation events in previously studied cases. Here we reported a paternity case with a mismatch in locus D18S51. Examination of 38 autosomal STR loci revealed no mismatches, and the paternity index is up to 2.68e+11. These results suggested that the alleged father is the biological father of the child. However, the genotypes of the alleged father, the mother and the child in D18S51 locus were 14/23, 15/16, and 15/20, respectively, which did not support the paternity relationship between the father and the child. It is doubtless that the allele 15 in the child was inherited from the mother, and therefore, allele 20 was a mutated allele originating from either allele 14 or 23 of the alleged father. Since the higher of the mutation steps, the less likely the mutation would happen, it is more likely that the D18S51 allele 20 of the child was inherited from the putative father's allele 23 with a three-step mutation.

138. MUTATION RATES FOR SHORT TANDEM REPEAT LOCI IN ECUADORIAN POPULATION

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Short tandem repeats (STRs) are sequences that involve repetitive units of 1-6 bp. For STR it has been reported mutations rates from 10-6 to 10-2 nt per generation, having the highest mutation rates the dinucleotide STRs, whereas the tetranucleotides are 50% lower. For forensic purposes, knowing the specific mutation rate for each locus on a particular population improves the calculations and also the interpretations of the data. More than 300 mutations were identified in STRs contained in PowerPlex 16, PowerPlex Fusion, GlobalFiler and VeriFiler in more than 13.000 paternity testing since 2013 to 2017 in the Genetic Laboratory of Cruz Roja Ecuatoriana. Moreover, between the identified mutations more than 51 were from maternal, more than 226 of them were from paternal source and more than 51 were undeterminable. Additionally, the highest mutation rate was observed in FGA and D12S391. The observed mutational behavior have important consequences in forensic DNA analysis like the definition of exclusion criteria in paternity testing and the correct interpretation. Thus, here we present the first report of STR's mutation rates for Ecuadorian population.

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139. RESULTS OBTAINED IN FIVE YEARS IN A PATERNITY TESTING LABORATORY IN MEXICO

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Reports from national and international organizations provide information on the state of the relationship testing community [1,2]. However, these reports do not include laboratories from Mexico and other Latin American countries. Therefore, we describe our findings in 3005 paternity cases analyzed during a period of five years in a Mexican paternity testing laboratory. Motherless cases were the most frequent type (77.27%), followed by trio cases (20.70%), the remaining 2.04% included different reconstruction cases. The non-paternity prevalence was 29.58%, higher than the reported by the AABB [1] (24.12%), and also greater than previous estimates from Mexico based on classical genetic markers (2.3 and 11.8%) [3,4]. We detected 59 mutations, most of them one-step mutation (93.2%), and estimated the mutation rate for 18 different STR loci. Five triallelic patterns and 12 suspected null alleles were detected during this period. As expected, those Human Identification (HID) kits including more STRs detect more mismatches (exclusions) and lead to higher paternity indexes (PI) and paternity

probabilities (W). However, the Powerplex 21 kit (20 STRs) and Powerplex Fusion kit (22 STRs) offered similar PI (p=0.379) and power of exclusion (PE) (p=0.339) when a daughter was involved in the test. The lower PE of D10S1248 and D22S1045 included in the Fusion kit [5] seems to explain this finding for motherless cases. In brief, it was evident that 20 STRs allow solving successfully the large majority of motherless paternity tests. This report will be a useful as reference for the paternity testing activity in Mexico.

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140. ROUTINE IMPLEMENTATION OF NON-INVASIVE PRENATAL PATERNITY TESTING WITH STRS

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The analysis of cell-free fetal (cff) DNA in maternal plasma for the detection of aneuploidies is becoming standard clinical practice. At 9 weeks of gestation the average circulating DNA fetal fraction in maternal blood is 10%, thus rendering this DNA amenable to characterization by massive parallel sequencing (MPS). The cffDNA can also be studied to enable inferences concerning paternity to be made, thus avoiding the mild risks associated with invasive procedures (chorionic villus biopsy or amniocentesis) to obtain prenatal fetal tissue. SNPs were (and are) used as the initial markers to identify the paternal component in cffDNA although thousands of these must be analyzed given that the circulating DNA is a mixture. More recently a pilot study (1) employing STRs to analyze the cffDNA was reported. In this work we describe the use of semiconductor MPS to analyze 32 STRs present on the autosomes and sex chromosomes in cffDNA. To date we have processed more than 110 tests as routine casework. Cases involving the confirmation of paternity in which no genetic discrepancies were observed and where the paternal alleles were clearly evident in the cffDNA always resulted in paternity indices >1000. We discuss the main challenges which include confidently knowing the fetal fraction, and dealing with the STR stutters which can severely hinder the allelic designations in this context of mixtures.

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142. ANALYZING AN "OFF-LADDER" ALLELE AT DXS10135 FROM THE AGCU X19 STR KIT

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X chromosome STRs (X-STRs) have been proven to be an important tool for some complex kinship analyses in recent years. A novel 19 X-STRs multiplex system named as AGCU X19 STR Kit was developed and widely used for population genetics as well as forensic case works. In this study an "Off-ladder" allele between allele 21 and allele 21.1 at DXS10135 has been observed in AGCU X19 STR Kit. The "Off-ladder" allele was about 0.4 bases longer than the allele 21 of allelic ladder of DXS10135 in AGCU X19 STR Kit. To avoid DNA profile misunderstanding or mislabeling, "Off-ladder" allele at DXS10135 was analyzed by sequencing and detected with Investigator Argus X-12 Kit, respectively. Our results showed this "Off-ladder" allele was allele 21 at DXS10135. It was caused by inaccurate allele 21 of allelic ladder of DXS10135 in AGCU X19 STR Kit.

143. BRAZILIAN FEDERAL DISTRICT GENETIC PROFILE BASED ON X-STR DECAPLEX

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Genetic profile based on X chromosome markers are useful helping to solve forensic cases, for example deficient paternity cases (1). Additionally, can also be useful in population genetics (2, 3). Here we present a Brazilian Federal District X chromosome population profile and forensic parameters, based on the multiplex amplification of X-Decaplex (developed by Spanish-Portuguese Group International Society for Forensic Genetics (ISFG)). Sample is compound of 205 men and 210 women (625 alleles). Federal District showed the highest Brazilian allelic diversity for these markers, including the highest amount of private alleles, considering the Brazilian regions analyzed to this point. The European contribution to the genetic composition of Federal District was estimated in 61.9%, whereas African was 27.0% and Amerindian 11.1%. These estimates are similar to the ones obtained employing autosomic genetic markers. The cumulative powers of discrimination estimated was 0.99999999996 for women and 0.9999995 for men. This report also contribute to the Civil Police of Federal District genetic database.

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144. GENETIC ANALYSIS OF 19 X CHROMOSOME STR LOCI FOR FORENSIC PURPOSES IN FOUR CHINESE ETHNIC GROUPS

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A new 19 X- short tandem repeat (STR) multiplex PCR system has recently been developed, though its applicability in forensic studies has not been thoroughly assessed. In this study, 932 unrelated individuals from four Chinese ethnic groups (Han, Tibet, Uighur and Hui) were successfully genotyped using this new multiplex PCR system. Our results showed significant linkage disequilibrium between markers DXS10103 and DXS10101 in all four ethnic groups; markers DXS10159 and DXS10162, DXS6809 and DXS6789, and HPRTB and DXS10101 in Tibetan populations; and markers DXS10074 and DXS10075 in Uighur populations. The combined powers of discrimination in males and females were calculated according to haplotype frequencies from allele distributions rather than haplotype counts in the relevant population and were high in four ethnic groups. The cumulative powers of discrimination of the tested X-STR loci were 1.000000000000000 and 0.999999999997940 in females and males, respectively. All 19 X-STR loci are highly polymorphic. The highest Reynolds genetic distances were observed for the Tibet-Uighur pairwise comparisons. This study represents an extensive report on X-STR marker variation in minor Chinese populations and a comprehensive analysis of the diversity of these 19 X STR markers in four Chinese ethnic groups.

145. GENETIC CHARACTERIZATION OF FOUR COLOMBIAN POPULATIONS USING INVESTIGATOR® ARGUS X-12 KIT

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X-STR markers have been shown to be a powerful tool for solving complex cases of biological relations; their use increases the probability of solving difficult cases using only A-STR markers. The objective of this study was to analyze 806 men from four Colombian populations (Department of Antioquia (n = 202), Department of Cauca (n = 200), Department of Santander (n = 204), and Cundiboyacense Region (n = 200)). The analysis were made with the Investigator® Argus X-12 kit. Allelic and haplotype frequencies, pairwise linkage disequilibrium (LD), pairwise FST genetic distances were all calculated using ARLEQUIN software v3.5.2.2, and some basic parameters (DPm, DPf, MECd, MECt) of forensic interest were established for each population (1). No significant differences were observed between Cundiboyacense Region and Antioquia and Santander Departments. High combined discrimination power was obtained in all populations, both in men (> 99.99999%) and in women (> 99.99999999999), as well as a high combined mean exclusion chance in father/daughter pairs (> 99, 99%) and

father/mother/daughter trios (> 99.9999). This work is contributing to increasing the knowledge of both the 12 X-STRs included in the Investigator® Argus X-12 kit and in the populations genetics of the Colombian population studied.

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146. GENETIC CHARACTERIZATION OF THE POPULATION FROM MATO GROSSO STATE, BRAZIL, BY ANALYSIS OF 32 INSERTION/DELETION POLYMORPHISMS ON THE X CHROMOSOME

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The genotyping of insertion-deletion polymorphisms (InDel) has been gaining importance in studies of population genetics and human identification over the last few years, as well as the study of X chromosome markers. In the present study 290 unrelated individuals (193 males and 97 females) born in the Mato Grosso State, Brazil, were analyzed for 32 X-InDel markers, considering that the data of this polymorphisms in this population are still unpublished, and that these markers may also in the future assist the resolution of forensic cases in our country. In the analysis of forensic efficiency parameters, all the markers were efficient for the studied population. Female samples showed a genotypic distribution in Hardy-Weinberg equilibrium (p> 0.00156, after Bonferroni's correction). The cumulative discrimination power (PD) for women was 0.9999999999998 and for men 0.9999997. The power of exclusion (PE) was 0.999997 in trios and 0.9999 in duos. Linkage disequilibrium (LD) analysis revealed a significant association between some pairs of markers (MID356-MID357, MID3690-MID3719, MID3719-MID2089 and MID3703-MID3774). These results indicate that the 32plex system can be used for human identification and kinship tests in the studied population.

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147. GENETIC DATA OF 10 X-STRS IN A POPULATION SAMPLE OF THE TUNJA CITY, DEPARTMENT OF BOYACÁ - COLOMBIA

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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), especially when the A-STRs are insufficient, and in cases of kinship analysis when the offspring is a female. In this research, an analysis of 10 X-STRs: DXS6809, DXS7423, GATA172D05, DXS6789, DXS9902, DXS7132, GATA31E08, DXS7133, DXS9898, and DXS8378 was performed in a population sample of Tunja City, Department of Boyacá-Colombia. A total of 253 individuals not biologically related were analyzed, 141 men and 112 women. All individuals lived in Tunja - 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, 0,999996 and 0,9999998 respectively. They have a power of exclusion (PE), higher than 0,999 for both trios (father-mother-daughter) and duos (father-daughter). Finally, we performed a genetic distance analysis showing significant differences between this population, Latin American, and Iberian populations. The results of this study showed that the 10 X-STRs markers are suitable to be used in forensic genetics and that Tunja city population of the Department of Boyacá - Colombia is a genetically differentiated population. For this reason, is recommended the development of appropriate databases of genetic markers specifics for this population.

148. KBC X-FILING: STR-BASED ASSAY FOR PROFILING OF X-CHROMOSOME

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X-STRs profiling bear the potential for kinship analysis, particularly in complicated cases which needed complementary efficient analysis of other genetic markers.
The "KBC X-Filing" kit is a novel 5-dye multiplex kit, for amplifying 15 X-chromosome STR markers (DX1187, DXS10101, DXS10146, DXS7132, DXS7423, DXS10148, DXS10135, HPRT, DXS10074, DXS6803, DXS10134, DXS10103, DXS10079, DXS981, DXS378), AMXY and D21S11 in a single reaction. This autosomal marker is included as an internal control for avoiding sample mix-up when a large number of samples are analysed. X-STRs are located throughout the length of X-chromosome.
Due to the use of direct PCR reagents, dried blood samples (DNA banking card, etc.), root hair, nail, blood, and saliva can be added directly to the reaction.
More than 50 individual males and 100 individual females have been tested using this kit. Internal validation tests have been performed and reliable results were obtained.

149. KNOWING THE DEMOGRAPHIC HISTORY OF THE MEXICAN POPULATION FROM X-STRS INCLUDED IN THE INVESTIGATOR ARGUS X-12 KIT

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X-STRs has proved useful in complex relationship cases. However, it is also a convenient tool to know demographic changes and historical processes of a population [1]. Although, Mexican population has been largely studied with autosomal markers and Y-chromosome these reports do not include X-chromosome data [2,3]. Therefore, we analyzed a total sample of 482 unrelated Mexican women, including two mestizo populations (n = 144) and seven Amerindian groups (n = 338), additionally we included a European-pool as a reference. The results were compared with previous data obtained from other genetic systems. We initially determined the allele frequency and statistical parameters of forensic efficiency concerning the kit Investigator Argus X-12, but the main objective was know if this genetic system provides relevant information about changes in the size of the population along weather. It is noteworthy that for the three systems, the two mestizo groups displayed the greatest heterozygosity compared whit the rest of populations. Moreover, groups Maya and Huichol showed greater heterozygosity for X-STRs and A-STRs, this result is important for discussion purposes due to the fact that these two populations are characterized by having a close relationship to their mestizo neighbors. A test whit the program Bottleneck showed significant values for Nahua and Mestizo-Northwest populations that explains the recent expansion of the population. The analysis of the genetic structure showed three main components, concordant with previous reports with autosomes [2]. In brief, the X-STRs provide valuable information about the demographic processes that have occurred in the Mexican populations.

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150. MULTIPLEX PCR FOR 19 X-CHROMOSOMAL STRS IN CHINESE POPULATION

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Application of the short tandem repeats (STRs) loci located in the X chromosome has become an efficient tool in forensic kinship testing. The aim of this study is to develop a new multiplex that analysis of 19 X-STRs loci (DXS9895, GATA144D04, DXS10077, DXS10078, DXS10161, DXS10160, DXS981, DXS6800, DXS6803, DXS9898, DXS6801, DXS6799, DXS6797, DXS7133, DXS6804, GATA172D05, DXS8377, DXS10146, and DXS10147) simultaneously in a single reaction and to evaluate the allele and haplotype diversity of this multiplex system in the Chinese Han population. In addition, the sensitivity test showed a complete genotyping profile with DNA as low as 0.25ng. The results indicate that the new 19 X-STRs multiplex system may provide high polymorphism information and could be used as a supplementary tool for kinship testing as well as X chromosome genetic mapping studies in China.

151. MUTATION RATE OF X-STRS FROM THE INVESTIGATOR ARGUS X-12 KIT IN ARGENTINE POPULATION

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The analysis of X-chromosomal markers can be important in different situations where the application of autosomal and Y- chromosomal STR markers are not sufficient to solve the cases.
Currently, the Argentine population lacks a representative database on X-chromosomal markers, regarding allele and/or haplotype frequencies, and mutation rates.
The absence of this information represents an important limitation for their routine use in laboratories, preventing the achievement of a quantitative, statistically supported evaluation.
In order to estimate mutation rates for the twelve X-chromosomal markers included in the Argus X-12 kit, 346 father-daughter duos were genotyped.
The samples were selected from all provinces of Argentina and the biological relationship of paternity was previously confirmed by the analysis of autosomal STR markers for all duos, for which likelihood ratios higher than 106 were achieved.
A total of 22 mutations over 4,152 allelic transmissions were observed at DXS7132, DXS10134, DXS10079, DXS10146, DXS10101, DXS10103, DXS10074, DXS10148 and DXS10135 loci.
The overall X-STR mutation rate observed was 5.3x10-3 (95% CI, 3.3 x 10-3 - 8.0 x 10-3) and all the genotypic configurations were explainable by the gain or loss of a single repeat.
Finally, it should be noted that the overall mutation rate observed in this work resulted higher in comparison with some other reports, likely due to only father-daughter duos had been considered. Indeed, these findings are in agreement with previous works suggesting higher mutation rates for males, due to the higher number of germ-line divisions they experience.

152. RECOMBINATION OF 19 X-STR LOCI IN CHINESE HAN THREE-GENERATION PEDIGREES

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The use of X chromosome STRs is very efficient for determining kinship in some complex paternity testing or forensic cases. Currently, a novel kit of 19 X-STR loci (DXS8378, DXS7423, DXS10148, DXS10159, DXS10134, DXS7424, DXS10164, DXS10162, DXS7132, DXS10079, DXS6789, DXS101, DXS10103, DXS10101, HPRTB, DXS6809, DXS10075, DXS10074 and DXS10135) was developed. The aim of this study was to investigate genetic linkage and recombination fractions of these 19 X-STR loci in 36 three-generation families of the Chinese Han population. Linkage analysis and calculation of

recombination between pairs of markers were carried out. Our study showed that these 19 markers could be grouped into constituting 6(presumably) independent linkage groups. The 3 recombination events between the adjacent loci of the linkage groups and 52 recombination events between the adjacent linkage groups were observed. The 4 mutations were found in the 4 loci. In conclusion, this study indicated that the multiplex system of 19 X-STR loci can be used as a useful supplementary method in complex kinship analysis.

153. X CHROMOSOME STRS: SEGREGATION AND GENETIC TRANSMISSION ALONG THE MATERNAL LINE

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Paternity trio cases can most easily be solved through autosomal STR markers alone, while paternity testing regarding duos involving a daughter, or more complex pedigrees, could gain from X-chromosomal testing, which allows to complement information acquired from the autosomes when there is a need for additional statistical strength.
X-chromosomal STRs analysis may be useful especially in complex pedigree testing, in deficiency paternity cases or in personal identification, when additional relatives are needed.
The usefulness of X-chromosome analysis is already proved when father/daughter relationship is questioned thanks to its specific inheritance pattern.
However, in order to verify the usefulness in complex pedigrees, it is necessary to define the segregation and the genetic transmission of this chromosome from mother to daughters and sons.
Therefore, to evaluate the consistency of 12 X-STRs loci included in the Investigator Argus X-12 Kit (Qiagen) (DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148 and HPRTB), X haplotypes in one or two generations (male siblings, mother / father with daughters, mother / father with sons) samples were compared.

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154. 350-YEARS OLD REMAINS IN WARSAW – TODAY'S REMINISCENCE OF XVII CENTURY WARS

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The XVII century in Poland was unfortunately extremely rich in conflicts: Muscovite–Lithuanian Wars, Cossack rebellions, Polish-Turkish Wars and Polish-Swedish Wars1, commonly known in Poland as the "Swedish Deluge" named after a novel by a Polish Nobel prize winner, Henryk Sienkiewicz. After over 350 years, what was believed to be the skeletonized remains of Swedish soldiers from that period of

time were found in the country's capital – Warsaw. To confirm or exclude the thesis, genetic profiling had to be performed, with the special focus on lineage markers. Teeth were taken from all 14 skeletons and after sample preparation, DNA was isolated with both phenol-chloroform and magnetic particles method. Autosomal STRs were amplified with GlobalFiler® (Thermofisher) and Investigator® 24plex QS (QIAGEN) PCR amplification kits. After obtaining Y chromosomal STRs with the use of Yfiler® Plus (Thermofisher) and PowerPlex® Y23 (Promega), Y-STR haplogroups of the individuals were estimated by Whit-Athey's algorythm2 and YHRD database, where possible. Mitochondrial DNA HV1 and HV2 fragments were sequenced and based on those results, mitochondrial haplogroups were estimated by the EMPOP database. The use of Next Generation Sequencing provided yet another tool for bioancestry estimation, based on Illumina's ForenSeq Signature DNA Prep Kit panel B, by sequencing with the use of MiSeq FGx Reagent Kit, on MiSeq FGx System, followed by analysis with ForenSeq Universal Analysis Software. Results obtained during the study confirmed the assumption about Northern-European origin of the individuals.

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155. A MULTIPLEX ALLELE-SPECIFIC PCR SYSTEM CONTRUCTED WITH SNP-STR LOCI

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Nowadays STR databases have come to a considerable scale, which makes STR become and will remain a main stream genetic marker in forensic science for years. In the process of STR profiling, amplification disequilibrium caused by different DNA content in the initial stage of PCR would lead to phenomenon like drop-in and drop-out that will affect profiling outcome, especially in the situation of mixed-DNA and low-copy DNA1. To overcome such negative phenomena in STR genotyping, we proposed that allele-specific PCR (AS-PCR) may reduce amplification disequilibrium in the initial stage of PCR. In the present study, we constructed a one-step SNPSTR system. Compared with Mountain's classic SNPSTR system2, it needs less human resource, takes less operation time, and thus has a better usability. Our results suggest it can reduce the amplification disequilibrium phenomena and may help the analysis of DNA mixtures. Meanwhile, the most of the STR loci we used to construct the AS-PCR panel are chosen from the markers that are using in most of the forensic criminal DNA database and are compatible with the current STR detection system.

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156. A PRIORI PROBABILITIES IN Y23 MIXTURE ANALYSIS

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When Y-STRs are used in the investigation of sexual offences, it is not uncommon to observe mixed profiles from two or more male individuals. If the haplotype of a person of interest is fully represented in the mixed profile then clearly that male could have been a contributor to that mixture.
The evaluation of the weight-of-evidence in such cases is more complex than in autosomal STRs (where the loci are inherited independently). Methods for calculating a likelihood ratio for a Y-STR mixture have been published [1]. However, these methods require complex mathematics to perform.
To further our understanding of the significance of a match within a Y-STR mixture we have devised a non-contributor experiment on simulated mixtures derived from published Y23 haplotypes from 19,630 different men [2] to estimate the average probability of a random inclusion (Pi).

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157. A STUDY OF THE GENETIC DIVERSITY IN THE HEZE HAN POPULATION USING A NOVEL GENOTYPING SYSTEM BASED ON 24 Y-CHROMOSOMAL STR LOCI

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We established a 24-plex Y-STR typing system by including the 13 Y-STR loci used in AmpFLSTR® Yfiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA) and 11 additionally selected Y-STR loci that significantly increased power of discrimiation . We applied the system to genotype 195 unrelated Chinese Han males , resulting in 195 distinct haplotypes. The diversity observed across loci ranged from 0.0885 (DYS645) to 0.9646 (DYS385a/b). , while the haplotype diversity was 1 based on the 24 Y-STR loci. The ultra-high differentiation power of the genotyping system has great potential in forensic applications.

158. AFRICAN L0A MTDNA HAPLOGROUP IN REPUBLIC OF MACEDONIA

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The distribution of mtDNA haplogroups in Macedonia is similar as in other countries of Balkan peninsula, with the most frequent European haplogroup H, than clusters U,T,J and K observed with intermediate frequency (1). Because Macedonia is a multiethnic country there are also some particular haplogroups specific for ethnic groups such as M5a1 for Romanies or H12 for Albanians (2).But till now there are no published data for presence of any of the African haplogroups in this small south-eastern European country. Presence of Sub-Saharan mtDNA haplogroup L0a in an orthodox Macedonian family with all maternal ancestors and descendants can be explained with later arrival of Sub-Saharan individuals in Europe and their dispersal throughout Europe.

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159. AN UNEXPECTED CASE IN THE PREHISTORY OF THE IBERIAN PENINSULA: BIOGEOGRAPHICAL ORIGIN ANALYSIS THROUGH MITOCHONDRIAL DNA

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We have determined the mitochondrial haplotype (A73G T16172C C16223T G16257A C16261T) on a Bronze Age Individual from an archaeological site located in Alaejos (Valladolid, Central Spain). This haplotype is typical from N9a2'4'5 mitochondrial haplogroup, which is nowadays widely distributed in the Asian region. Here, we compare and discuss the frequency of this haplogroup in nowadays and in ancient human populations, especially in contemporaries of the individual under study.
The biogeographical analysis has been carried out by amplifying two overlapping fragments of the Hipervariable Region I (HRVI) and other two of the Hipervariable Region II (HRVII) of the mitochondrial DNA (mtDNA). Moreover the mtDNA haplogroup was assigned by the analysis of mtSNPs in coding regions [1]. Furthermore, the experimental process was performed by duplicate to obtain a reliable haplogroup. Along the all process, ancient DNA authenticity criteria were observed [2].

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160. ANALYSIS OF DEGRADED CASEWORK DNA BY REDESIGNING A MINI Y-STR MULTIPLEX

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As partial genetic profile from degraded DNA samples of a case limiting the application of Y-STRs in forensic sciences, a new multiplex mini Y-STR typing was developed to obtain more useful information and complement current commercially available multiplex kits. The multiplex mini Y-STR amplification including three Y-STR loci (DYS385a/b, DYS389 II, and DYS392) was performed to generate amplicons smaller than those of PowerPlex® Y system over 140bp by redesigning primers. Allelic ladders for three mini Y-STR loci were created using a dilution of allelic ladders from the PowerPlex® Y system. A sensitivity test using serially diluted standard 9948 male DNA showed that three mini Y-STR loci were reliable at template concentrations as low as 0.25ng. By comparing of the multiplex mini Y-STR amplification and PowerPlex® Y system with referenced DNA samples, the results indicated stable concordance in genotyping. And then the successful analysis for the degraded DNA samples from the forensic case produced complete genetic profiles with these three loci. So the multiplex mini Y-STR typing combining the aforementioned commercially available kit increased chance of gaining of 16 Y-STR loci profiles within some core loci in Y Chromosome Haplotype Reference Database from forensic casework sample. It is a alternative strategy for recovering locus-specific information from degraded to redesign the suitable mini Y-STR complement besides new commercial Y-STR kits.

161. ANALYSIS OF MATERNAL LINEAGES IN THE MALTESE POPULATION

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Situated in the centre of the Mediterranean, Malta has a rich demographic history (1). Malta was a small island population with ~20,000 inhabitants (1000AD). It saw the settlement of Sicilians and Italians together with an influx of other population groups over the years which caused a population admixture. The Maltese population increased exponentially in the last 1000 years reaching 450,000 today. The Maltese are not an entirely homogenous population due to founder effects which shaped the gene pool (2).
Maltese mitochondrial DNA (mtDNA) data is not publicly available. A new high-quality mtDNA control region dataset was setup to evaluate Maltese mitochondrial lineages. Samples and ancestry data were collected from 300 unrelated individuals from the Maltese Islands. This collection is archived in the Population Bank of BBMRI.mt. The entire mtDNA control region was amplified with two PCR primers and sequenced with a minimum of four sequencing primers using the EMPOP protocol (3) and followed forensic quality guidelines (4). The haplotype and haplogroup data was compared to other population groups in the Mediterranean basin to define matrilineal relationships.

This research provides an insight into the genetic origins of the Maltese. The dataset could be used as first national reference database for mtDNA applications in forensic and missing persons casework and population genetics studies.

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162. ASSESSMENT OF MTDNA LINEAGES OF FILIPINOS FOR FORENSIC APPLICATIONS

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In most forensic casework, the highly-discriminative short tandem repeat (STR) analysis is used in profiling and identifying individuals and kinships of interest. However, in challenging forensic casework i.e. identifying highly-degraded remains, human mitochondrial genome (mtDNA) typing has been utilized as an alternative or complementary option. Its high sensitivity, stability, and the unique approach of typing distant maternal relatives allows identification where routine STR testing cannot. In this study, we sequenced the mtDNA control region (nucleotide positions 16 024 – 16 569, 1 – 576) of 141 unrelated Filipino male individuals and analyzed them with previously published sequences. Results show the high diversity of the Filipino population as shown by the low random match probability (1.51%), high haplotype diversity (98.49%) and high nucleotide diversity (0.014161±0.007100). We used spatial PCA and DAPC to plot the distribution of the haplogroups in the archipelago and assess the level of resolution contributed by each of the three mtDNA hypervariable regions (HVI, HVII, HVIII). Multi-dimensional scaling plot using pairwise F_{ST} revealed minor clustering with other Southeast Asian populations, which indicate presence of admixture between populations. This study aims to expand the Philippine database in terms of sample and genetic coverage, and explore the use of mtDNA as investigative leads for future casework applications.

163. BIOLOGICAL KINSHIP ANALYSIS IN EXTREMELY CRITICAL SAMPLES: THE CASE OF A SPANISH NEOLITHIC NECROPOLIS

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It is common in archeology and some forensic cases to make assumptions about the relationship between persons buried in the same grave, or in distinct burials but in the same cemetery. However, there is no objective criterion to make such suppositions.
This study aims to investigate the biological relationship among five Neolithic individuals (3954-3380 cal BC) (1) from the Can Gambús-1 necropolis (Barcelona, Spain) as well as, their possible mitochondrial ancestry, and the determination of their molecular sex, for a subsequent comparison with the available anthropological information. From at least 48 tombs, five skeletons were selected, from five individual graves, selecting from each one three integral teeth, without cavities or caries.
To carry out this study, the samples were analyzed by two distinct extraction methods, one destructive and other non-destructive (2), and to determine a presumptive (biological) kinship between the buried individuals, small length nuclear DNA markers were considered, as well as a mitochondrial DNA analysis, in order to determine their maternal lineage.
The samples were extremely degraded, requiring several attempts to achieve sequences with a standard quality, without double peaks or other artefacts.
Despite the degraded state of the samples, it has been possible to analyze the mitochondrial information and to determine that the studied individuals had a European ancestry, and were not maternally related, since they did not share the same mitochondrial haplotype.

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164. CHINESE POPULATION GENETIC SUBSTRUCTURE USING 23 Y-CHROMOSOMAL STRS

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China, as the largest national database within the YHRD (Y chromosome haplotype reference database), has repeatedly been a research hotspot in the field of genetics, archaeology and molecular anthropology. In the present study, 23 Y-STR loci were used to investigate genetic relationships among 29 Chinese populations (6029 individuals). Pairwise genetic distance (R_{st} value) was calculated based on haplotype frequency distributions by the Analysis of Molecular Variance. Multidimensional scaling (MDS) and phylogenetic analysis were performed on the online tool and Mega 6.0, respectively. Tibetans (R_{st} = 0.1601 ± 0.0421), Shuis (R_{st} = 0.0934 ± 0.0327), Uyghurs (R_{st} = 0.0791 ± 0.0459) and Zhuangs (R_{st} = 0.0790 ± 0.0404) showed the high genetic distance with other populations and kept relatively isolated with other reference populations in the MDS plots and neighbor-joining tree. Additionally, genetic distinction among same ethnicities (Han and Hui) distributed in different administrative divisions was also explored and no significant differences were identified.

165. COMBINING THE EVIDENTIAL VALUE OF MULTIPLE PARTIAL DNA PROFILES USING A LOWER BOUND FOR THE LIKELIHOOD RATIO

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In forensic casework, it is common that multiple partial DNA profiles are obtained in a single case. For the evaluation of such evidence, it is necessary to consider the possibility that they originated from different sources. One could report the evidential value of the separate profiles by computing likelihood ratios for them separately. However, by doing so, information regarding the conditional dependency structure is lost and the potentially difficult step of combining them is left to the trier of fact. Especially in situations where some, but not all, of the observed loci in the separate profiles overlap, a combined evaluation could be substantially more informative. In such situations, it is not unrealistic that the separate evidential values are not optimally combined by the trier of fact. When forensic scientists believe that their knowledge regarding the dependency structure between pieces of evidence is lost by presenting the likelihood ratios separately, one should strive to combine this evidence before it is sent to the trier of fact. For a combined evaluation, the prior distribution for the number of distinct sources of the DNA profiles becomes part of the likelihood ratio. Generally, prior probabilities are beyond the scope of expertise of the forensic specialist and are left to the trier of fact to decide. We propose a lower bound likelihood ratio approach for such situations that is independent of the prior distribution. Using a set of examples, we present how this method can be applied in practice

166. COMPARISON OF 2 MTDNA HAPLOTYPES FROM CZECH EXCAVATION SITE WITH THE RESULTS OF MITOCHONDRIAL DNA STUDIES ON EUROPEAN NEOLITHIC AND MESOLITHIC INDIVIDUALS

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The aim of the study was to compare the mtDNA haplotypes (haplogroups H and W) obtained from 2 young females buried within a Neolithic circular enclosure (rondel) dated to the Stroked Pottery Culture (4934-4970 cal.BC (95,4 %), and 4650-4462 cal.BC (95,4%)) at Kolín (Czech Republic) with the results of the scientific studies on Neolithic and Mesolithic skeletal material. We collected 166 mtDNA literature sequences and data about the age of the specimen, location of the excavation site (Denmark, France, Germany, Hungary, Italy, Poland, Spain, and Sweden), D-loop mutations, haplogroups, sequencing primers and methods used. The frequency of the haplogroups (H=35%, U= 22%, K=13,5%, T=7,7%, J, N, X = 5%) from this data set differs from the current European population but is in a good shape with the previous findings that identified mtDNA haplogroups H and U being typical for early farmers and hunter-gatherers (1). Haplogroup W, which has never been found in ancient DNA samples in Europe

before the Neolithic, is connected with early/middle Neolithic expansion (2).

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167. COMPREHENSIVE MUTATION ANALYSIS OF 53 Y-STR MARKERS IN FATHER-SON PAIRS

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The knowledge of mutation rate at Y-STR markers is essential in forensic casework, especially kinship genetic studies. In the present study, one hundred father–son pairs from Chinese Han population were typed with 53 Y STR markers (38 conventional markers and 15 rapidly mutating markers). A total of 27 mutations were detected in 6201 allele transfers. All were one-step mutation. The average estimated mutation rate of 15 rapidly mutating (RM) Y-STRs was 1.05×10^{-2} (95% CI 5.45×10^{-3}-1.39×10^{-2}), while the average mutation rate of 38 conventional Y-STRs was 1.46×10^{-3} (95% CI 3.07×10^{-4}-2.59×10^{-3}). In summary, the average mutation rate of 15 RM Y-STRs seems to be seven times more than that of 38 conventional Y-STRs.

168. CONSTRUCTION AND FORENSIC GENETIC CHARACTERIZATION OF ELEVEN AUTOSOMAL HAPLOTYPES CONSIST OF TRI-ALLELIC INDELS

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Insertion-deletion polymorphisms (Indels), which combine the advantages of both short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs), are suitable for parentage testing. To overcome the limitation of poor polymorphisms of diallelic Indels, we constructed a set of haplotype with physically linked multi-allelic Indels. Candidate haplotype blocks were selected from 1000 Genome database, and should fulfill the following criteria: (i) minimum allele frequency (MAF) ≥0.10 in the population of Southern China (CHS); (ii) exits in non-coding region; (iii) the physical distance between two candidate Indels should less than 500bp; (iv) an allele length variation of each Indel should be 1-20bp; (vi) on different chromosomes or chromosomal arms, and physical distance larger than 10Mb when the two haplotype blocks on the same chromosomal arm; (vii) should not be located in recombination hotspot. A multiplex system with 11 haplotype markers, including 22 Indel loci, which distributed over 10

chromosomes was developed. To validate the multiplex panel, the haplotype distribution in a set of pedigrees was investigated. Our results demonstrated the haplotype consist of multi-allelic Indel markers shown higher polymorphism than single Indel locus, and could provide supporting information for forensic application.

169. DEVELOPMENT OF RM Y-STR HAPLOTYPE DATABASE FOR ARABIAN PENINSULA POPULATIONS

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Y-chromosomal short tandem repeats (STR) analysis is a valuable tool which has been broadly applied in forensic caseworks especially sexual assaults cases, population studies, genealogical research and kinship analysis. Since the outbreak of rapidly mutating Y-STR (RM Y-STR) loci, these markers were proven to have significantly superior resolution of male lineage differentiation. Therefore, in order to estimate the statistical weight of RM Y-STR haplotype match in the populations of the Arabian Peninsula, a database of detailed haplotypes is required. Forensic laboratories from different Arab's countries collaborated to study and develop an Arabian Peninsula quality controlled RM Y-STR haplotype database. 1228 unrelated, male individuals from six countries; United Arab Emirates, Kingdom of Saudi Arabia, State of Kuwait, State of Qatar, Kingdom of Bahrain and Oman were collected. A subset of these samples was analyzed using Yfiler® allowing the comparison between RM-YSTR and the conventionally used Y-STR markers. Forensic parameter including gene diversity, haplotype diversity, match probability and discrimination capacity were estimated for each population and Arab populations overall. AMOVA analysis was performed between Arab's populations and other populations. Network analysis was also performed using 11 single allelic markers in order to represent the substructure between these populations.

170. DNA ANALYSIS OF LINEAGE MARKERS FROM SKELETONS FROM A MASS GRAVE RELATED TO THE BATTLE OF REICHENBERG IN 1757

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During a rescue excavation in October 2011, archaeologists discovered a mass grave with 10 male

individuals. The skeletons should belong to victims of the battle of Reichenberg between the Austrian and Prussian armies on April 21, 1757. Several bones of the skeletons were covered with a blue colored encrustation. Initial DNA analysis failed due to strong inhibition. Chemical analysis of the bluish encrustation indicated the presence of the iron phosphate mineral vivianite (Fe3(PO4)2.(H2O)8). DNA extraction and the inhibitor removal was performed according to the procedure described previously (1). We managed to obtain Y-chromosome and mtDNA haplotypes from all tested individuals. Resulting haplotypes were compared to the contemporary population from the vicinity of the excavation site as well as to the publicly available databases.

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171. DNA IDENTIFICATION OF 10TH CENTURY FEMALE SKELETON FROM A PRAGUE CASTLE APPARENTLY BELONGING TO A MEMBER OF RULING PRZEMYSLIDS DYNASTY

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The start of dynasty dates back to the 9th century when Przemyslids ruled a territory around Prague, populated by the Czech tribe of the Western Slavs. The first historically-documented Przemyslid Duke was Borivoj I (†888-890).
The aim of this work is to present the results of DNA identification of 10th century female skeleton from a Prague Castle apparently belonging to a member of ruling Przemyslids Dynasty. The skeleton was buried next to the skeletal remains that can be assigned to Boleslav I (†972) or Boleslav II (†999). DNA analysis yielded partial autosomal (MiniFiler, Thermo Fisher Scientific, USA) and X-STR profile (Argus X-12, Qiagen, Germany) and mtDNA haplotype (HVR1 16017-16569, HVR2 001-577). DNA typing data did not match to any results obtained so far from the Prague castle burials. Therefore we performed a comparison of mtDNA haplotype with publicly available databases as well as with mtDNA haplotypes of other European dynasties.

172. EVALUATION OF A REAL TIME PCR ASSAY FOR HUMAN MITOCHONDRIAL DNA QUANTIFICATION OF MINERALIZED SAMPLES

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Analysis of human Mitochondrial DNA (mtDNA) is a reliable alternative for challenging forensic

samples, but its success depends on the amount and integrity of the targeted DNA on the sample. We previously develop a human specific mtDNA quantification assay in order to better guide downstream applications. In this work, we present the results from analysis of 15 highly decomposed skeletal remains and hypervariable regions HVI and HVII full sequences usually are obtained when approximately 500 or higher copies of mtDNA are inputted per reaction (CE analysis).. The feasibility of the assay has been demonstrated by the minimization of the consumption of judicious samples and by optimized levels of DNA template to be amplified during mtDNA analysis, increasing the chances of success at analysis.

173. FORENSIC EVALUATION OF 27 Y-STR HAPLOTYPES IN A POPULATION SAMPLE FROM NIGERIA

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Nigeria is a country located in Gulf of Guinea, in West Africa, facing the Atlantic Ocean. It is one of the most populous country in the world. There are more than 500 ethnic groups inhabiting the territory, representing a high diversity of languages and cultures. In this study, we collected 142 samples from the three largest ethnic groups in Nigeria: Hausa, Igbo and Yoruba. These samples were typed for the 27 Y-STR loci included in the Yfiler® Plus kit. A total of 140 different haplotypes were found, with two haplotypes shared by 2 individuals. The haplotype diversity was 0.9998 (± 0.0009), slightly higher than that obtained with the 17 Y-STRs from the Yfiler kit (0.9992 ± 0.0010). The Yfiler kit also showed an increased number of shared haplotypes, with 4 being shared by 2 individuals and one by 3. The average gene diversity over loci was lower in Nigeria than in the U.S. population groups reported in Yfiler® Plus kit User Guide. The DYS391, DYS390, DYS438, DYS392, DYS437 and DYS533 showed low diversity values (GD ≤ 0.35). Comparisons between Nigeria and five populations in East Africa, the only African data available for the Yfiler® Plus markers, revealed significant differences among all populations (p-values < 0.00005). The smallest F_{ST} was found with Kenya (F_{ST}=0.025), followed by Ethiopia (F_{ST} =0.105). As expected, distances with Djibouti, Eritrea and Somalia were higher that 20%, since our sample are from Bantu speakers, not represented in these population sample.

174. GENETIC CHARACTERIZATION OF 27 Y-STR LOCI IN THE QINGHAI TIBETAN POPULATION

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Qinghai province is China's largest Tibetan community except Tibet. In this work we investigated the distribution of 27 Y-STR loci（DYS456, DYS576, DYS570, DYS481, DYS387S1, DYS627, DYS458, DYS460, DYS437, DYS439, DYS392, DYS385a/b, DYS393, DYS391, DYS390, DYS635, DYS449, DYS533, DYS438, DYS448, DYS389I/II, DYS19, Y-GATA-H4 and DYS518) in Tibetan in Qinghai, China. Samples of 529 unrelated Tibetan male individuals were amplified using the SureID® 27Y Human STR Identification Kit(HEALTH Gene Technologies, Ningbo). The PCR products were detected on an ABI 3130 genetic analyzer and analyzed with GeneMapper® ID v3.3 software. A total of 507 different haplotypes were found in the 529 unrelated Qinghai Tibetan males and the haplotypes diversity was 0.9998. The one marker that usually presents a high diversity in European populations showed the lowest value of diversity in the Qinghai Tibetan population, namely the DYS391(GD=0.3154). On the other hand, the DYS385a/b showed the highest diversity(GD=0.8968). The study shows us that the 27 Y-STR loci in Tibetan populations of Qinghai province have a high degree of genetic polymorphism and ethnic characteristics. Therefore, the results derived from this study can not only provide valuable information about the Y chromosome variation in Qinghai Tibetan population but also demonstrate their usefulness in forensic casework and paternity

175. GENETIC CHARACTERIZATION OF FOUR BRAZILIAN STATES WITH 27 YFILER®PLUS MARKERS

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The Y-STRs have high mutation rates, being useful to discriminate unrelated males. They are widely used in paternity and forensic investigations, and to study the recent history and migration movements of populations. Recently, the Yfiler®Plus was released to increase discrimination inside populations, by adding 10 Y-STRs to the previous kit version (YfilerTM). The genetic composition of Brazil is known to vary through 5 geopolitical regions: South, Southeast, North, Northeast and Central-west. Therefore, for this study, samples selected from Maranhão (n=114), Espírito Santo (n=76), São Paulo (n=126) and Rio Grande do Sul (n=202) were characterized for the Yfiler®Plus markers, in order to evaluate if diversity increases with the enlargement of the YfilerTM markers set, as well as to see if significant differences exist among populations. Genetic differentiation analysis did not reveal statistically significant differences in the Yfiler®Plus haplotype composition of the studied samples ($FST \leq -0.0001$; $P \geq 0.7099$). High diversity values were observed in all samples for both YFiler ($HD \geq 0.9957$) and Yfiler®Plus ($HD \geq 0.9992$) haplotypes. Inside populations, when considering the Y-STRs from the YFilerTM kit, 20 haplotypes were shared by two individuals (2 from Espírito Santo, 7 from Maranhão, 11 from Rio Grande do Sul). These numbers decrease to 1 in Espírito Santo, 5 in Maranhão and 8 in Rio Grande do Sul, when using the full set of 27 Y-STRs. The Yfiler®Plus demonstrated an increased discrimination power in comparison to the YFilerTM, being suitable for forensic applications in the studied populations from Brazil, for which data were not yet available.

176. GENETIC DIVERSITY OF 71 Y-CHROMOSOMAL BIALLELIC MARKERS IN THE HAN POPULATION LIVING IN SOUTHERN CHINA

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Polymorphic markers located on Y chromosome represent an important complement for special kinship cases. We analyzed 71 Y-chromosomal biallelic markers including 65 SNPs and 6 insertion/deletion events in a sample of 201 unrelated Han males living in Southern China. The panel was amplified in three multiplex reactions–32plex, 31plex and 8plex following standard protocols for iPLEX chemistry. Comparisons were then carried on with our previous data from Northwestern Han populations. As results, 66 markers were observed to be polymorphic (MAF≥0.01), of which 19 loci (rs17323322, rs17316543, rs11096433, M119, rs2032678, rs17276338, M117, rs17316007, M134, rs52812045, rs9786394, rs17269396 , rs2267801 , M122, rs17316592, rs17269816, rs17269928, rs17276358 and rs13447361) were high informative (GD≥0.3). A total of 67 haplotypes were identified, of which 37 haplotypes were unique, 12 haplotypes were found in two individuals and the remaining 18 were shared among 3 to 29 individuals. The haplotype diversity was estimated to be 0.9535. Significant differences were found between Southern and Northwestern Han population at 32 loci. The results showed that joint usage of multiplex amplification and MALDI-TOF-MS could have advantages in screening and genotyping biallelic markers. In addition, the panel could provide supplementary information for forensic identification, but the significant differences observed within Han populations should be taken into account in casework.
Keywords MALDI-TOF-MS, Y-SNP, Y-InDel, Forensic science

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177. GENETIC POLYMORPHISMS OF 25 Y-STR MARKERS IN JAPANESE

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We investigated allele and haplotype frequencies of 25 Y-chromosome short tandem repeat (Y-STR) markers (DYS576, DYS389I, DYS635, DYS389II, DYS627, DYS460, DYS458, DYS19, GATA-H4, DYS448, DYS391, DYS456, DYS390, DYS438, DYS392, DYS518, DYS570, DYS437, DYS385, DYS449, DYS393, DYS439, DYS481, DYF387S1 and DYS533) in 1299 Japanese individuals using the Yfiler® Plus PCR Amplification Kit. DNA samples were extracted from liquid blood collected from 1299 unrelated Japanese males living in five major islands of Japan (Hokkaido, Honshu, Shikoku, Kyushu and Okinawa). A total of 1294 haplotypes were identified, of which 1290 haplotypes were unique. The haplotype diversity was 0.999993, and the discrimination capacity was 0.996151. The gene diversity values ranged from 0.2370 (DYS391) to 0.9498 (DYS385). Thirteen different microvariants called as off-

ladder alleles were detected (namely at marker DYS576: the allele 16.2; DYS627: the alleles 22.1 and 23.2; DYS518: the alleles 36.2, 37.3 and 45.2; DYS570: the allele 18.3; DYS449: the alleles 29.1, 29.2, 30.2 and 35.1; DYF387S1: the alleles 37.2 and 38.3). No allele was detected at DYS437 in one sample, at DYF387S1 in one sample and at DYS448 in 12 samples.

178. GENETIC PORTRAIT OF THE HAZARA POPULATION OF PAKISTAN WITH 26 Y-STR LOCI

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The Hazara population of Pakistan is ethnically associated with the Hazaras of neighboring country Afghanistan. Hazara individuals have typical Mongolian facial features and they claim to be descendants of Genghis Khan's army in the first quarter of the thirteenth century AD. In this study, we genotyped 153 unrelated Hazara males living in Quetta, Balochistan, Pakistan, for a total of 26 Y-chromosomal STR loci. Each Y-STR locus showed diversities ranging from 0.241 to 0.773, and overall discrimination capacity (DC) was 87.5% with 126 different haplotypes using the PowerPlex Y23 loci. By adding three Y-STRs (DYS388, DYS449 and DYS460) to the PowerPlex Y23 loci, the DC increased to 92.36% while the number of different haplotypes increased to 133. The Hazaras of this study showed significant differences from other local populations of Pakistan as well as neighboring populations, while showing genetic affinities with Kazakhs and Mongolians. Our results thus contribute to understanding the potential forensic usefulness of the 26 Y-STRs studied and also shed light on the population history of Pakistani Hazaras.

179. HAPLOTYPE ANALYSIS OF THE POLYMORPHIC 24 Y-STR MARKERS IN SIX ETHNIC POPULATIONS FROM CHINA

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Twenty-four Y-STR loci were analysed in 1446 males from the following six Chinese ethnic populations: Guangxi Han (n=600), Gin (n=161), Maonan (n=135), Miao (n=186), Zhuang (n=226) and Yao (n=138) using the AGCU Y24 STR amplification kit. The lowest estimates of genetic diversity (below 0.5) correspond to markers DYS391 (0.4006), DYS438 (0.4300), and DS388 (0.4907), and the greatest diversity corresponds to markers DYS385a/b (0.9636) and DYS527a/b (0.9439). Moreover, there were 1331 different haplotypes identified from the 1446 total samples, of which 1233 were unique. Notably, we observed shared haplotypes between the four ethnic populations (Maonan, Miao, Zhuang, Yao ethnic population), except between the Guangxi Han and Gin population. The estimated overall haplotype diversity (HD) was 0.9997. A multidimensional scaling (MDS) plot based on the genetic distances between populations demonstrates the genetic similarity of the Maonan, Miao, and Zhuang populations with genetic distance below 3.0. No substructure correction is required to estimate the rarity of a haplotype comprising 24 markers. In summary, the results of our study indicate that the 24 Y-STRs have a high level of polymorphism in these six Chinese ethnic populations and could therefore be a powerful tool for forensic applications and population genetic studies.

180. HAPLOTYPE DIVERSITY OF 23 Y-CHROMOSOME STR LOCI IN POPULATION OF PERSUMED TO BE NORTH KOREAN MEN

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North Korea has been divided with South Korea over 70 years. Despite important geographical position of the Korean Peninsula in East Asian human migration and expected accumulation of differences between North and South Korean gene pools, DNA profiles of North Korean had never been reported in Y-chromosome STR Haplotype Reference Database(YHRD; https://yhrd.org). In this study, a total of 105 haplotypes were analyzed on PowerPlex Y23 loci. All haplotypes were unique and the haplotype diversity and discrimination capacity were both 1.0000. The gene diversities were ranged from 0.3145 to 0.9718. In 16 Y-filer loci and 8 minimal loci, 92.3% and 80.0% of matched haplotypes were determined haplogroup O, Southeast or East Asian type. The multidimensional scaling (MDS) plot and neighbor joining (NJ) tree based on R_{st} values with other 11 Eurasian populations were indicated that they have closest relationship with South Korean, following Japanese, Thai and Vietnamese. In addition, calculated p-value with South Korean was 0.1582, indicating that they are still one genetic population.
Keywords: North Korea, PowerPlex Y23, YHRD, Genetic distance.

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181. KINSHIP INFERENCE FOR MALES WITH IDENTICAL Y-STR PROFILES USING WHOLE GENOME SNP DATA PROVIDES A DEEPER UNDERSTANDING ABOUT THE LEVEL OF COANCESTRY IN THE SWEDISH MALE POPULATION

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Analysis of Short Tandem Repeat markers on the Y chromosome (Y-STRs) can be performed for various forensic applications such as linking a suspect to a crime scene, extracting the male component of a male-female mixture in rape cases and for resolving kinships involving hypothesis of relationships

in the same paternal lineage. Due to the haploid status and the inheritance pattern of the Y chromosome, the interpretation of a Y-STRs match could be complicated since male relatives may share, for several generations, an identical Y-STR profile. This fact, in combination with an increased level of population substructure, generally makes the evidential value of a Y-STRs match limited compared with a match with autosomal STRs. In order to obtain a deeper understanding about the relatedness among randomly "unrelated" male individuals in the Swedish population and also to be able to improve the biostatistical interpretation, we have performed whole genome SNP analysis (>600.000 autosomal SNPs) on male individuals having identical 17 loci Y-STR profiles. For each pair of individuals we have estimated the degree of kinship and made comparisons within and between groups of men having identical and non-identical Y-STR profiles, respectively. This study show that even though identical Y-STR profiles are shared, there is no evidence that these individuals are related in a higher degree compared with randomly related male individuals in the Swedish population.

182. LINKING THE Y-CHROMOSOMAL HAPLOTYPE FROM HIGH MEDIEVAL (1160-1421) SKELETON FROM PODLAZICE EXCAVATION SITE WITH LIVING DESCENDANTS

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The presented work is a partial result of our study on cemeteries located in marginal areas of the populated regions in Central Bohemia. The excavation site of our interest is located within the range of the deserted male Benedictine cloister in Podlazice. The anthropologic examination indicates a rather high ratio of children's and female graves. It seems plausible that the monastery cemetery was also used by community of the near-by village. DNA analysis on the skeletons from this site includes autosomal STR typing, Y-chromosomal typing and mtDNA typing. We managed to link one of the retrieved Y-haplotypes with a group of individuals bearing the surname Dusanek (Duschanek). One of them even confirmed that his ancestors lived close to Podlazice cloister from where they moved after the Thirty Years' War (1618-1648) to another area of Bohemia. The differences in haplotypes (genetic distances) between the skeleton and the closest matches are 15 and 16 Y-STRs out of 17 tested using the Y-filer kit (ThermoFisher Scientific). This finding match our expectations based on the Y-STR mutation rate.

183. MASSIVELY PARALLEL SEQUENCING OF 24 Y-STR LOCI IN THAI POPULATION

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Massively parallel sequencing technology has been demonstrated as a potential tool for genetic studies and recently, for forensic context. ForenSeq™ DNA Signature Prep Kit (illumina, Inc.) has been

designed exclusively for forensic DNA analysis that autosomal STRs, X-STR, Y-STR and identity SNPs can be sequenced simultaneously. These informative genetic data empower the achievement of global DNA database presenting in autosomal STRs and Y-STR. The Y-STRs comprised of 9 core loci, 2 multi-copy loci and other 13 Y-STR loci. Here, we examined 24 Y-STR of 182 Thai male population that originate from Central, Western and Southern regions of Thailand. In this study, the average gene diversity (GD) of this kit is 0.7137. The lowest gene diversity is 0.4187 from DYS391 locus and DYS385a-b shows the highest gene diversity (0.9582). One hundred and seventy-seven haplotypes have been observed and therefore, these 24 Y-STR markers would be beneficial for forensic investigation, paternity test and genealogy study in Thai population.

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184. MASSIVELY PARALLEL SEQUENCING OF THE WHOLE MITOCHONDRIAL GENOME FROM HUMAN HAIR SHAFTS

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Mitochondrial DNA (mtDNA) with a significantly high copy number offers advantages in forensic casework that mainly contains low-quantity or no detectable nuclear DNA, such as hair shed. MtDNA analysis has been focused on the control region, but a recent development of massively parallel sequencing (MPS) has made whole mitochondrial DNA sequencing easily accessible. Here, we analyzed the whole mtDNA sequences of head hair obtained from 20 Korean males using MPS method. DNA was extracted from 2 cm of hair shafts that had been stored at room temperature for more than a year. Extracted DNA was subjected to PCR amplification of whole mtDNA using the Precision ID mtDNA Whole Genome Panel kit. Barcoded MPS libraries were prepared and pooled libraries were sequenced on an Ion S5 System. The obtained whole mtDNA sequences were aligned to the rCRS and compared with those obtained from bloods and buccal swabs of the same individual. In addition, we determined the mtDNA haplogroups of each sample based on the observed haplogroup-specific mutations, and scrutinized the presence of point heteroplasmy. This whole mtDNA sequencing using MPS method will provide a useful perspective to hair shed in forensic DNA analysis.

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185. MITOCHONDRIAL DNA AND Y-CHROMOSOME DIVERSITY IN THE CLOUD FOREST AREA OF NORTHEASTERN PERU

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Our research focuses on the origin and population history of the human communities that inhabit the cloud forests of northeastern Peru, with both contemporary and ancient DNA data. Here we report preliminary 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 populations: Chachapoya, Huancas, Jivaro and Cajamarca. Both lineage marker data showed high levels of genetic diversity for all populations, especially in the Chachapoya (e.g. h=0.9974±0.0032/ Y-chromosome). Interestingly, this population also shows signatures of population expansion for both markers such as 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. Additionally, in Neighbor-Joining trees, the Chachapoya assumes a basal position among most South American populations. This evidence points to a complex and distinctive past demographic history in the region.
To deepen our understanding on various demographic processes in this area, located in the juncture of two major ecosystems, the Andes and the lush Amazon, we have produced a subset of whole mtDNA (N=162) and Y-SNP (N=110) data, which is currently being analyzed in a population genetic framework. This will allow us e.g. to make haplogroup assignments more accurately, but more importantly, it will serve to test several hypotheses such us the existence of a constant effective population size (Ne) through time in the Chachapoya. This and other questions of population genetic interest, in a region of South America little explored until now, will be addressed in this presentation.

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186. MTDNA CONTROL REGION ANALYSIS OF NORTH BRAZILIAN POPULATION

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South America's demographic complexity has been historically influenced by population interactions such as the European migration and African slavery trade, besides Native Americans groups previously settled in the territory. In Brazil, colonization occurred alongside different historical events at the

beginning of the 16th century which intensely contributed to the genetic admixture. This study aims to enlarge on the databases with matrilineal lineages of Brazilian population. We analyzed the mtDNA control region of blood spots from 116 individuals belonging to the North region of the country which includes seven of the 23 Brazilian States: Acre, Amazonas, Amapá, Pará, Rondônia, Roraima and Tocantins. All samples were processed by direct amplification and the extension products were performed on ABI Prism® 3500 with the BigDyeTM Terminator v3.1 kit. The haplogroups were assigned using the online website EMPOPver.3 after the assembly and comparison to the revised Cambridge Reference Sequence (rCRS) using the SeqScape® Softwarev2.5. Our data revealed higher rates for the Native American ancestry (57.8%), followed by African (36.2%) and European (6.0 %) proportions. The most common haplogroup was L (36.2%) and the haplogroup C (25.9%) was predominant among Native Americans. In addition, self-declared data from the latest census published in 2010 by the Brazilian Institute of Geography and Statistics (IBGE) shows that the North region population is composed by: indigenous (37.4%), brown (12.9%), black (7.3%) and white (4.1%) people. Considering the lack of mitochondrial lineages data, further studies should be conducted in order to fully elucidate anthropological and population genetic pattern across the country.

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187. MTDNA STUDY OF GUINÉ-BISSAU IMMIGRANT POPULATION LIVING IN LISBON

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Guiné-Bissau is a country on the west coast of African Continent. Before the arrival of Europeans and until the XVII century, the territory was part of the Gabu kingdom, Mali Empire.
In the past decades the number of immigrants in Portugal has grown and Guinean immigrant community is one of the largest immigrant communities. Most of those immigrants are based in Lisbon metropolitan region.
Over the last decades, mtDNA has become an important genetic marker in human and population evolution studies, as well as in forensic and clinical case studies. Maternal inheritance, high copy number, lack of recombination and high mutation rate are characteristics that makes this genetic marker useful in studies of human origin and evolution.
The non-coding mtDNA region presents high genetic variability and the haplotypes obtained through the study of this region fall into haplogroups with specific polymorphisms shared by all individuals with a common ancestor.
We studied 127 blood samples from Guinean immigrants living in Lisbon. mtDNA control region was amplified and sequenced between positions 16024 and 576, using two sets of primers - L15971/H016 and L16555/H639 -.
The study of the control region revealed high genetic diversity within Guinean immigrants, with a high frequency of unique haplotypes. Most of the obtained mitochondrial DNA sequences belong to African haplogroups.

As the result of the integration of African immigrants in Lisbon population we will have, in a near feature, individuals borned in Portugal with Portuguese nationality exhibiting mtDNA haplotypes typical of regions of the African Continent.

188. MTDNAMAP: GEOGRAPHIC REPRESENTATION OF MTDNA HAPLOGROUPS

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Mitochondrial DNA is inherited maternally and is thought to be evolved stepwise from one population to another population in the history of mankind. Haplogroup for any mtDNA provides us a solution for the logical classification of the mitochondrial DNA based to established phylogenetic principles. There is huge amount of scattered mtDNA sequence data from different global and regional populations. It demands a professional platform for representation of data to draw meaningful and simple-to-understand information about mtDNA distribution. Here, mtDNAMap provides geographical representation of mtDNA haplogroups' frequencies in various populations all over the world according to their present day reported locations. It is haplogroup frequency database of different populations calculated from the published data using their reported valid mtDNA sequences. Publicly available MtDNA sequences, processed through mtDNAprofiler for SNP determinations based on revised Cambridge Reference Sequence and followed by Haplogrep 2.0 for the determination of the haplogroups on the basis of most updated Phylotree version-17, are graphically represented on dynamic map in the form of frequencies. mtDNAmap provides the open access to whole or part of published high-quality curated data. The tool is not only useful for researchers from forensic and anthropology backgrounds but also to general public.

189. MUTATION RATE ANALYSIS FOR 13 RM Y-STRS BY USING A NOVEL MULTIPLEX PCR SYSTEM IN SHANDONG HAN POPULATION

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Abstract: The investigation of the mutation rates of the 13 rapidly mutated（RM）Y-STRs in Shandong Han population was performed to verify the mutation characteristic and application among Chinese population. We analysis 2880 allele transfers in 180 father-son pairs by using a novel multiplex PCR system which was developed by our research team. Based on our data,we can differentiate 19.44% of 180 father-son pairs. The haplotype of four father-son pairs showed inconsistent at two loci.39 mutation events was observed. The mutation rates of 13 RM Y-STR ranged from 0.0000 (95%CI, 0.0000-0.0203)

to 0.0500 (95%CI, 0.0231-0.0928), and the average mutation rate was 0.0135(95%CI, 0.0096-0.0185) . We observed that the top three RM Y-STR in Shandong Han population were DYF399S1abc, DYF403S1b2 and DYS570. All mutations discovered in this study are one-step mutation, and a mutation rate of repeat loss:gain was 1.17:1.

190. MUTATION RATE AT 13 RAPIDLY MUTATING Y-STR LOCI IN THE POPULATION OF SERBIA

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Recently, the interest of the forensic community has been focused on new Y-chromosomal short tandem repeats (Y-STRs), termed Rapidly Mutating Y-STRs (RM-YSTRs), which is able to differentiate between close male belonging to the same paternal lineage due to their high mutation rates. In this study we have estimated a mutation rate for 13 RM-YSTR in 85 pairs of male relatives in the population of Serbia. We analysed 74 father-son pairs, and 11 twin pairs, to evaluate the capacity of distinguishing between male subjects within a single lineage. Each father-son couple was previously confirmed by autosomal STRs testing (AmpFℓSTR® Identifiler Plus™ kit, Applied Biosystems) with paternity probability ≥99.99% and also confirmed monozygotic or dizygotic twins. Results showed that, in the 74 father-son pairs 23 mutations were detected of which 22 were one-step mutations and 1 was two-step mutation, while in the 11 twin pairs 1 mutation was observed in one dizygotic twin pair. Five father-son pairs were found to have mutations at two loci, while one pair at four loci. Overall, the most mutable markers were DYF399S1, DYF387S1, DYF403S1a and DYS612. Our findings are encouraging and concur with previous studies showing that by RM-YSTR typing the discrimination power of male relatives could be considerably increased in comparison to every YSTR markers commonly used in forensic genetics.

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191. MUTATION RATES AND SEGREGATION DATA ON 16 Y-STRS: AN UPDATE TO PREVIOUS GHEP-ISFG STUDIES

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The increasing relevance of human Y-STRs in forensic science demands reliable estimates of their mutation rates. Therefore, a collaborative study was carried out by the Spanish and Portuguese working group of the International Society for Forensic Genetics (GHEP-ISFG) in the interest of extending the data on Y-chromosomal short tandem repeat (Y-STR) mutation rates. Sixteen Y-STRs were considered in the analyses: DYS456, DYS389I, DYS389II, DYS390, DYS458, DYS19, DYS385, DYS391, DYS392, DYS393, DYS439, DYS635, DYS437, DYS438, DYS448, GATA H4. Among the sample of 1601 father-son duos analyzed, 46 mutations were observed, 45 of which were a single-step change and 1 was a double-step change. A total of 27 repeat losses were observed against 18 gains, with a ratio of 1:1.5. Eleven pedigrees showing double alleles at the Y-STR loci DYS19, DYS391, DYS439 and DYS448 without allelic discrepancy between the father-son duo were also observed. This new data was added to the previous studies from the GHEP-ISFG working group [1,2], totalizing 49,039 allelic transmissions (varying between 2302 and 5946 per locus). The average mutation rate across all 16 Y-STRs loci was 2.04x10-3 (95% CI 1.66x10-3 – 2.48x10-3). The average mutation rates per marker varied between 5.05x10-4 (95% CI 1.04x10-4 – 1.47x10-3) at DYS385 and 6.09x10-3 (95% CI 3.67x10-3 – 9.49x10-3) at DYS439.

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192. MUTATIONAL ANALYSIS OF 27 Y-CHROMOSOMAL STRS PERFORMED ON 85 MALES FROM ONE DEEP-ROOTED SERBIAN PEDIGREE

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Y-chromosome microsatellites are widely used in forensic analyses to characterize paternal lineages of unknown trace donor, as well as paternal kinship analyses, population history studies and evolution. In order to increase resolution of paternal lineage, large effort have been made in the past years to improve commercially available Y short tandem repeats (Y-STRs) kits by including greater number of rapidly mutating (RM) STRs. Most previous studies on mutation rates included only father–son transmissions or pedigrees related by small number of meiosis. Here we present a study performed on 85 males from large pedigree, using 27 Y-STR markers that included also six RM Y-STRs. The analysis was performed with PowerPlex® Y23 System and AmpFLSTR®Yfiler™ Plus kits, as well as non-commercial RM Y-STRs. Ancestor Y-STR haplotype was successfully reconstructed, so we were able to visualize changes in founder haplotype and to access mutation rates for each STR in 366 meiosis that took place. Of a total 48 mutation events, 46 were one-step, 1 was two-step and 1 was three-step mutation. We observed mutations in DYS627 RM (5/48), DYS570 RM (5/48), DYS518 RM (4/48), DYS449 RM (4/48), DYS576 RM (4/48), DYF387S1 RM (1/48), but also in loci that are not rapidly mutated DYS456 (5/48), DYS439 (5/48), DYS549 (4/48), DYS458 (3/48), DYS481 (2/48), DYS389I (2/48), DYS391 (2/48), DYS389II (1/48) and DYS437 (1/48). As the aim of Y-STRs analysis is to differentiate unrelated men in a population, reliable locus-specific mutation rates are necessary and could be achieved by deep rooted-pedigree analysis like this.

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193. POPULATION ANALYSIS OF AFRICAN Y-STR PROFILES WITH UNIQ TYPER™ Y-10 GENOTYPING SYSTEM

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The Y-STR multiplex in [1] has been developed as a commercial prototype, UniQ TyperTM Y-10, with PCR reagents modified from its original version, and a dye-matrix designed and constructed for its new dyes [2].
A large screening of Southern African populations, 679 Bantus (249 Tswanas from Botswana, 95 Zulus, 199 Xhosas, 42 Vendas and 94 Pedis from South Africa) (FDL unpublished data and [3]), 202 admixed Cape Coloured, 93 Indians and 101 Afrikaners was conducted in Southern Africa with this genotyping system. A total of 983 unique haplotypes were detected. Bantus showed the lowest DC (90.13%), followed by Indians (95.7%), and both Coloured and Afrikaners with 93%.
Standard Fst analysis showed significant differences in between all pairwise comparisons between Bantus, Afrikaners, Coloured and Indians. AMOVA using an Fst distance matrix indicated that 4.9 % of the genetic variation was explained by differences among Bantus, and 4.55 % due to differences among groups.
Factorial correspondence analyses of haplotypes showed an important clustering structure likely determined by haplogroup ancestry. The analysis and identification of haplogroups is in progress.

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194. POPULATION DATA AND FORENSIC GENETIC EVALUATION WITH THE YFILER® PLUS PCR AMPLIFICATION KIT IN A LITHUANIAN POPULATION

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The analysis of Y-STR markers is used to identify victims from natural disasters, rapes or murder, sexual assault cases, moreover it provides information about genetic diversity and can determine genetic differences in population. Yfiler™ Plus PCR Amplification kit (Thermo Fisher Scientific, Waltham, MA, USA) has 6-dye technology and can amplify 27 Y-STR loci including 7 rapidly mutating markers(RM Y-STRs) and 11 mini-STRs. This study reports on the genotyping of 254 individuals from Lithuania were analyzed using Yfiler™ Plus kit. Forensic and population genetic parameters were calculated using relative computer equipment, statistical approaches and computational tools. Population comparison with other European populations from YHRD were performed. Our results revealed that 27 Y-STR loci provides high haplotype diversity, high discrimination power and is an effective tool in forensic genetics. After comparing data of allelic percentage distribution in each examined Y chromosome locus and genetic diversity in these microsatellite areas between Lithuania's and other world's populations, Lithuanian population is not very polymorphic or unique and isolated from other populations. When comparing genetic distancies, an observation was made, that Lithuanian population with its Y chromosome genetically is the closest to Polish and Russian populations. Inconclusion, the Yfiler™ Plus kit is very useful in daily casework in forensic laboratories.

195. POWERFUL USE OF NEW TOOLS FOR MITOCHONDRIAL DNA INVESTIGATION

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Mitochondrial DNA is a powerful investigative tool for forensic science, with particular application to scenarios where nuclear DNA is either absent or severely degraded. The advances in massively parallel sequencing technology have provided new opportunities to enhance this mitochondrial DNA analysis, both by simplifying the analysis of whole genome sequences and by improving the sequencing success rate for compromised samples. We present here the mitochondrial sequencing results obtained when amplifying the control region in multiple overlapping fragments as implemented in the PowerSeq

Mito Nested System Prototype kit developed by Promega, with sequencing performed on a MiSeq. To demonstrate the utility and reproducibility of this method, we present the results of a sensitivity study along with the application to a case involving ancient hair samples.

196. RAPIDLY MUTATING Y-STRS POPULATION DATA IN THE POPULATION OF SERBIA AND HAPLOTYPE PROBABILITY ASSESSMENT FOR FORENSIC PURPOSES

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Y-chromosomal short tandem repeat (Y-STRs) markers are widely used in casework due to their paternal inheritance, which can help to resolve kinship cases, and identify male components in male/female mixtures. Recent research with Rapidly Mutating Y-STRs (RM Y-STRs) have shown that these markers provide substantially higher haplotype diversity and haplotype discrimination capacity in worldwide populations when compared with the Y-STRs commonly used in genetic forensics. The aim of this study was to develop an allelic frequency database for the population of Serbia in order to evaluate the resolution power of 13 RM Y-STRs. A total of 279 unrelated males from the population of Serbia were typed with 13 RM Y-STRs markers: DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526a/b, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627. A high Y-STR haplotype diversity was found (0.999897) in our sample. As expected, the RM Y-STR loci showed high genetics diversity (GD) values (>0.70) in the Serbia population. The highest GD was observed for the locus DYF399S1 (0.991), followed by loci DYF403S1a (0.976), DYF387S1 (0.907) and DYF404S1 (0.899). In addition, AMOVA test was performed between population of Serbia in comparison to several worldwide population data sets. Based on the results of this study, the RM Y-STR markers showed remarkable haplotype resolution power in the population of Serbia, high genetics diversity and, therefore, demonstrating their usefulness in forensic identification cases.

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197. THE COMPARISON OF Y-CHROMOSOMAL HAPLOGROUP PREDICTORS

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Predictors of Y-chromosomal haplogroups (Y-Hg) are widely used tools for results where defining SNP information is not available. The aim of this study was to compare the results of Y-Hg prediction using different predictors. We used Y-chromosome DNA typing data from 342 unrelated individuals where we obtained Y-STR haplotype together with haplogroup defining SNP. Samples were genotyped for the non-recombining region of the Y chromosome with the TaqMan technology in a hierarchical manner for a set of variable number of SNP markers and for six indels. All individuals were also typed for a set of 19 STRs using two different multiplexes: 17 STRs were amplified with the Yfiler kit (Applied Biosystems) and two additional STRs, DYS426 and DYS388. Resulting Y-STR haplotypes were subsequently entered into 5 different Y-haplogroup predictors (Vadim Urasin, Nevgen, Hapest, Jim Cullen, Felix Immanuel). We constructed the median joining network for our haplotypes and compared the haplotypes with YHRD database records. We also evaluated the impact of number of used STRs (12 vs. 17) on the resulting Y-haplogroup prediction.

198. THE FEW AND FAR BETWEEN—Y-SNP HAPLOGROUPS FOUND AT LOW FREQUENCIES IN THE TYROLEAN ALPS OF AUSTRIA

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In an attempt to improve our understanding of the present day Y-chromosomal landscape of the Tyrolean Alps in Austria we previously determined both the Yfiler STR haplotypes and the major Y-SNP haplogroup affiliations of 3711 resident men. In total, nearly 97% of these Y chromosomes were assigned to haplogroups R1b (42.3%), I (16.0%), G (11.2%), R1a (9.8%), J (9.4%), and E (8.1%). The follow-up work presented here focuses on Y chromosomes attributed to haplogroups known for being rare in Western and Central Europe, such as Q-M242 or G-L91. To gain additional phylogenetic resolution, a two tier strategy was employed. First, the 17-locus Yfiler haplotypes of the queried samples were utilized for in silico haplogroup prediction by means of freely accessible online computer software (bit.ly/2namnUj, bit.ly/2nabEZN). In a second step, these predictions were scrutinized by Sanger type sequencing of Y-SNP markers being informative in the respective phylogenetic context. Generations-of-residence as well as kinship filtering were based on the genealogical information provided by the voluntary study participants.

199. THE FINDING OF DISACCORD IN HAPLOGROUP PREDICTION BY ONLINE SOFTWARE IN A FATHER-SON PAIR

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The Y-chromosome haplogroup usually determined by the patterns of single nucleotide polymorphism (SNP) refers the geographic or ethnic origin of unknown samples. Recent studies have showed that the haplotypes defined by Y short tandem repeats (STRs) may also be used to predict haplogroup status. The development of online software for Y-haplogroup predictions provides numerous benefits to the forensic application. When samples from a father-son pair in a case were genotyped using the PowerPlex® Y23 system, mutation at DYS385ab locus (13,14 to 13,15) was observed. Then haplogroup prediction based on their different haplotypes was conducted with Whit Atheys' haplogroup predictor (http://www.hprg.com/hapest5/hapest5b/hapest5.htm.), and disagreement between the father and the son (Q and O3, respectively) was detected. After performing necessary validation using SNP analysis, consistent haplogroup of the father-son pair was assigned. Although Y-STR typing seems more widely used in forensic casework and convenient to predict haplogroup with online tools, our finding suggested that the occurrence of errors in Y-chromosome haplogroup prediction should be cautious. As increasing the number of STRs employed to predict the haplogroup, correcting inaccurate prediction due to mutation is required by means of precise Y-SNP phylogenetic data.

200. THE IMMIGRANT POPULATION FROM MOZAMBIQUE IN LISBON: UPDATED MITOCHONDRIAL DNA PORTRAIT

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Since the end of the 1970s Portugal had a role in the migratory movements, becoming a destiny for immigrants of a wide range of nationalities, especially from the African continent. According to statistical data, until the end of 2014, there were approximately 3000 Mozambican immigrants living in Portugal and from those, more than a half living in Lisbon metropolitan region.
Mitochondrial DNA identical sequences are shared by matrilineal inheritance. Along with the lack of recombination, it's possible to trace the ancestral origin of each population and his evolutionary history. However, not only in evolutionary and population studies but also in forensic genetics, mtDNA is an important tool.
The aim of our study is the genetic characterization of Mozambican immigrants living in Lisbon in order to emphasize their genetic variability contribution to Lisbon population.
Blood samples were collected from 83 Mozambican immigrants residents in Lisbon. Control region of the mtDNA was amplified using two pairs of primers: L15971/H016 and L16555/H639. The amplified products were sequenced by BigDye®Terminator v.3.1 Cycle Sequence (AB) and the sequenced products were detected in a sequencer Genetic Analyzer 3130 (AB). Finally the results were analysed by Sequencing Analysis v.5.2 software and also compared with rCRS using SeqScape v.3 (AB) software. The haplogroups were determined based on Phylotree, build 17.
A wide range of haplotypes belonging to L, R, J, H, U and M haplogroups were founded. The highest incidence was observed for the L haplogroup, often pointed as characteristic Sub-Saharan region, where Mozambique is framed.

201. THE Y CHROMOSOME PORTRAIT OF THE MALTESE

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A study of the Y chromosome to assess Mediterranean genetic structure, which included 90 Maltese samples, revealed that the Maltese cluster with the Central-East Mediterranean group with Southern Italy, Turkey and Cyprus in the same category (1). Another study on the Y (n=187) describes Maltese ancestors as Phoenician (2). Autosomal STR analysis of the Maltese (n=157) showed a close genetic relationship of the Maltese with the Sicilians (3). No Maltese Y chromosome data is currently available on the YHRD and this study aims to analyse the male lineages of the Maltese population with high resolution.
A panel of 23 Y-STR markers were used to assess Y chromosome variation in 300 unrelated males from the Maltese Islands. The PowerPlex® Y23 System is a five-dye multiplex that combines 17 Y-STR loci with 6 rapidly mutating (RM) Y-STR loci. The high gene diversities of the RM Y-STRs increase the ability to distinguish male relatives. This study followed the ISFG guidelines on the use of Y-STRs in forensic analysis.
Genetic and haplotype diversity, discrimination capacity and match probability were calculated. Maltese Y-STR haplotypes were compared with others from countries bordering the Mediterranean. This dataset will be made available on the YHRD and will provide a local male lineage reference population database valuable for both forensic and population genetics applications.

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202. TRACING ARAWAKAN SOUTHERN DISPERSAL: CLUES FROM MITOGENOME SEQUENCING OF SOUTHERN AMERINDS

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Arawakan is one of the most widespread linguistic families in South America, reaching as far as the Caribbean shorelines and the Brazilian Pantanal. Linguistic Bayesian analyses described a possible origin of the language in western Amazon, succeeded by several migrations into southern Amazonia, along the Amazon River until the Atlantic shore of French Guiana, northward to the Caribbean coast

and within northwestern Amazonia. The Arawakan groups not only share linguistic traits, but also show certain characteristics that distinguish them from the rest of the Amazonian groups. Arawakan are considered a high culture, as they show highly complex societies with established ruling and religious hierarchies. The Arawakan are also acknowledged by their ancient agricultural habits, which is reflected in the proto-Arawakan vocabulary. In fact, their strong agricultural traditions are thought to have led to an increase of population density that might have caused the successful migrations. Here we have analyzed mitogenomes from different Arawak groups and a historically relevant Panoan tribe. A particular haplogroup B2 lineage was found along the Ucayali River in Peru and the Brazilian Pantanal of Mato Grosso do Sul and has been deeper explored. As common commercial trade routes were established alongside the Ucayali and Madre de Rios from Peruvian Lowlands until Bolivian Beni, our study might help clarifying the southern migration of Arawakan groups following the same riverine route until reaching the wetlands of Brazilian and Paraguayan Pantanal. A more refined mtDNA characterization of such isolated groups can also improve the accuracy of forensic databases.

203. Y CHROMOSOME ANALYSIS OF EXTANT ARGENTINEAN MENNONITES

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Present day Argentinean Mennonites arrived to Argentina in the late XXth century, descending mainly from individuals living in colonies in Canada, México and Bolivia. In this study we analyzed Y-chromosome short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) in 12 males from the Argentinean community of Mennonites, 'La Nueva Esperanza'1,2. The analysis of the Y-chromosome haplogroup composition showed that all the Y-chromosome haplotypes observed in Argentinean Mennonites are of European ancestry, pointing mainly to the Netherlands as the population in Europe with the highest Y-chromosome affinity. Standard molecular diversity indices exhibit a much lower diversity than neighboring populations from Argentina or European populations, which is probably indicative of a moderate level of endogamy in the community. Thus, although the reduced size of the sample analyzed is a limitation, the results of this study indicate that Argentinean Mennonites do not show signals of Native American or African introgression and suggest that their Y-chromosome pool has been most likely inherited from their original homeland in Europe3,4. Ongoing mitochondrial DNA analyses will be of particular interest in order to confirm if Argentinean Mennonites were biologically permeable to other neighboring populations given that the Native American component in the mtDNA pool of admixed Americans is significantly higher than the one observed on the Y-chromosomes.

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204. Y-CHROMOSOMAL STR HAPLOTYPES IN A SAMPLE FROM MATO GROSSO, BRAZIL

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Objective- To characterize in the population of the State of Mato Grosso (Brazil) 23 Y-STR markers included in the PowerPlex Y23 System, Promega. Materials and Methods- A total of 200 unrelated individuals and residents of that state were enrolled in the study. DNA extraction was performed with Chelex 100, followed by amplification with PowerPlex Y23 Systems and capillary electrophoresis with 3500 genetic analyzer. Statistical analysis was performed with the Arlequin Software and Analysis Molecular Variance (AMOVA), available on the Y chromosome STR database (YHRD). Results- A total of 197 haplotypes were identified, 194 were unique and three were found in two individuals each, resulting in a high discrimination capacity (0.985) and haplotype diversity (0.9998) of the genetic system in the population studied. In addition, two intermediate alleles were genotyped for marker DYS385 (10.2 and 12.2) and four for DYS458 (16.2, 16.3, 17.2 and 18.2), which were also reported in other populations. Pairwise RST distances between the population of the Mato Grosso and other 30 admixed populations from Brazil, available in the YHRD, showed lower values of RST (-0.0059 to 0.0169) with non-significant p-value after applying the Bonferroni correction ($p < 0.0001$) reported by other authors in studies from central Brazil. Conclusion- The PowerPlex Y23 system proved to be highly discriminatory in the analysis of the State of Mato Grosso. In addition, for this system, the population studied does not have a genetic substructure in relation to the other admixed populations from Brazil not having a specific forensic database to this state.

205. Y-CHROMOSOME POLYMORPHISMS IN THE UNITED ARAB EMIRATES POPULATION

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This study analyzed Y chromosome of 345 Emiratis recruited from seven different Emirates in the UAE. The United Arab Emirates is located in the eastern part of the Arabian Peninsula, extends along part of the Gulf of Oman and the southern coast of the Arabian Gulf. We have evaluated the effect of geography on the distribution of genetic variability. The inter/intra group variation were analyzed. All the individuals were genotyped for 17 Y-STR markers. We have observed a total number of 124 alleles distributed across the 17 loci in the UAE population. The analysis of the allele frequency in the UAE population clearly shows that each locus has a predominant allele and most alleles are clustered over narrow range where 60-80% of individuals are sharing the same allele. We have observed 301 haplotypes of which 271 haplotypes are unique and 5 haplotypes are shared at least between two individuals. However, AMOVA analysis shows no significant inter-group variation and that most of

variation are within the population. Although the results cannot predict the genotypic variation manifested by 17 STR from other neighboring population, the UAE population show different allelic variation from the greater Middle Eastern region and Indian Sub-continent. This study is the most extensive study carried out on UAE population and provide the base-line study for further deep ancestry studies and for forensic science.

206. Y-STR HAPLOTYPES IN IRANIAN ETHNIC GROUPS

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Profiling Y chromosome is used in forensic genetics, migration studies, population genetics, and genealogy in all around the world. This study performed using “KBC 17-Plex Y-Filing” and “KBC 27-Plex Y-Filing” (Kawsar Biotech Company, Iran) in order to amplify 17 and 27 Y-chromosomal short tandem repeats (Y-STRs**.(**

To construct a representative Y-STR database of Iranian population; 445 individual males from Iranian ethnic groups (Kurd, Turk, Lor, Balouch, Sistani, Golestani, Mazni, Gilak, Fars, Khuzestani, Khorasani) were profiled. To increase the power of discrimination, by using “KBC 27-Plex Y-Filing” 10 other polymorphic Y-STR were studied for more than 100 individuals**.**

Obtained results of 17 loci were submitted to YHRD database (accession numbers: YA004237-44) and several intermediate alleles were observed which had not been reported yet. Null allele (AMXY and DYS458) were observed in 2 Balouch males, and after profiling his family members, these null alleles were detected in all 50 male members. DYS385 tri-allelic was detected in 4 males and duplication of DYS19 (5males) and DYS456 (1 male) were observed**.**

Analysis of molecular variance (AMOVA) reveals significant for genetic distances between the east and west of Iran. In addition, genetic structure analysis of Y-STRS showed a correlation between genetic distance and geographical distance. Although, several profiles from Khuzestan had a far distance with the other populations**.**

Iranian Y-STR profiles were compared with neighbor countries, the most similarity was observed between Iranian and Turkish males**.**

The results could be used as reliable references for forensic and population genetics studies.

Topic 04: New Technologies, DNA Typing Methods, Quality Control

207. COLOMBIAN RESULTS OF THE INTERLABORATORY QUALITY CONTROL EXERCISE 2015

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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 Interlaboratory Quality Control for the year 2015 are presented. The exercise consisted of one practical component, one theoretical mandatory component, and an optional theoretical component. Twenty laboratories participated, representing seven different countries of Latin America and the Caribbean. For the practical component each participant laboratory receive; (1) samples of blood, saliva, and 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 mandatory theoretical approach about a biological relationship of paternal grandparents – granddaughter case. In the last two components of the exercise, they had to submit only the calculations. 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 0.80% in genotyping, 1.0% nomenclature and/or format discrepancies and 3.8% non-reporting. On the other hand, 99.4% of the reported values were correct, only two values were not correct (0.6%) but were within the range of 5% of the correct value, being acceptable values within the rules of the exercise. This inter-laboratory exercise has become an important mechanism for quality assurance and ongoing training in the region.

208. EXPERIENCES IN ORGANISING OF PROFECIANCY TESTS SINCE SIX YEARS

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In Switzerland, it is mandatory for laboratories specialized in the field of forensic science to be accredited according to ISO17025. For quality assurance, the laboratories have to participate in four profeciancy tests every year. Since 2011, our laboratory is responsible for the organisation and preparation of two annual swiss interlaboratory profeciancy tests (CH-RV). Both profeciancy tests consist of a mandatory and an optional section. The obligatory part always contains between two and three different stains (single/mixture) and at least one buccal swab, which the laboratories have to analyse with their inhouse methods. In addition, the participants have to calculate the origin of the mixture with possible further interpretation of the results/stains. One of the two annual profeciancy tests requires additional interpretation of parentage. The optional part varies: it could range from analysing of

more difficult stains to theoretical tasks. Nowadays, nine laboratories participate regularly in the profeciancy test, some of those laboratories are departments of an institut of legal medicine and other laboratories are private enterprises. We will present our organisation of profeciancy tests for a small number of participants. The results will highlight the benefits and drawbacks, as well as challenges of small number profeciancy tests.

209. NEED FOR DEDICATED TRAINING, COMPETENCY ASSESSMENT, AUTHORISATIONS AND ONGOING PROFICIENCY TESTING FOR THOSE ADDRESSING DNA TRANSFER ISSUES

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Those authorized to report on the weights of DNA evidence are frequently called upon to address questions on DNA prevalence, transfer and persistence (DNA-PTP). Due to a lack of standardization, knowledge and training, these questions are often addressed poorly or inadequately, thus potentially adversely impacting justice outcomes. As the forensic community moves towards contemplating activity level assessments, it becomes incumbent on the provider to be well equipped to do so. The likelihood of alternative scenarios must be provided using data and methodologies that are valid both foundationally and as applied. The expert must be aware of the current knowledge of variables potentially impacting any assessment, including those involving DNA-PTP, and the associated limitations. We have conducted preliminary assessments of DNA reporting scientists on their general understanding of DNA-PTP and their ability to identify key factors that could impact transfer probabilities. Differences were observed between individuals in their level of comprehension of DNA-PTP. Apart from the need for further studies to validate the variables impacting DNA-PTP and the extent of their impact in different situations, we advocate that individuals utilizing this information should be specifically authorized to conduct such analyses and provide expert opinion in criminal investigations and legal proceedings relating to DNA-PTP. Furthermore, improvements are required in the foundational and ongoing training of these individuals. The setting of standards to be met by such training, how to test competency, and the availability of regular purpose-fit proficiency tests, requires urgent attention.

210. THE EFFECT OF EFFORTS TO REDUCE LABORATORY CONTAMINATION EVALUATED BY THE RESULTS OF MONTHLY ENVIRONMENTAL DNA MONITORING

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As the sensitivity of forensic DNA analyses has increased, there is also an increased risk of detecting a contamination in a DNA sample. The current sensitivity level in DNA analyses requires stricter

measures to prevent contamination. Background DNA present in the forensic examination environment (e.g. on equipment or bench tops used during examinations) creates a risk of contamination. During the past years the section of Forensic Biology at Oslo University Hospital has had a thorough review of the of the in-house laboratory environmental DNA monitoring program. This has led to new (improved) efforts to reduce contamination, such as changes in detergents used to clean laboratories and improved procedures in regards to laboratory behavior to avoid contamination. We present here the environmental DNA monitoring program used at the section of Forensic Biology at Oslo University Hospital. We also demonstrate, from the results of monthly environmental DNA sampling before and after changes were implemented, how the efforts to reduce contamination have had an effect on the potential level of contamination from the environment.

211. THE TRANSLATION OF ISO 18385:2016 REQUIREMENTS INTO THE PRODUCTION PRACTICE

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ISO 18385:2016 specifies requirements for the production of products used in the collection, storage, and analysis of biological material for forensic DNA purposes. The aim of this standard is to minimize the risk of occurrence of detectable human nuclear DNA contamination in products used by the global forensic community. ISO 18385:2016 excludes microbiological, animal, plant DNA and mtDNA testing. The Anex A of this standard specifies procedures for environmental monitoring of the production areas, validation of post-production treatments and the testing of final products.
There are several manufacturers offering forensic DNA grade products that comply to ISO 18385:2016 (e.g. Eppendorf, ThermoFisher Scientific, Promega, COPAN, Qiagen, MACHEREY-NAGEL, SceneSafe…). We have compared the particular procedures and protocols and suggest the best laboratory practice for the compliance testing of Forensic DNA grade products.

212. ACCURATE MEASUREMENT OF THE RATIO OF MITOCHONDRIAL TO NUCLEAR DNA BY DROPLET DIGITAL PCR

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When casework samples are either highly degraded or contain low amounts of genomic DNA, testing of mitochondrial DNA may be more advantageous. Six real-time quantitative PCR (qPCR) assays for mitochondrial DNA have been identified in the literature [1-6] and four were further optimized for droplet digital PCR (dPCR). While the previous assays were using dilution series of plasmids or oligos to establish standard curves in qPCR to quantify mitochondrial DNA, digital PCR does not require a standard curve.
Droplet digital PCR relies on the partitioning of the PCR reactions into an oil emulsion. This

partitioning allows the estimation of the number of accessible amplifiable targets without the need for an external calibrant. The use of multiple independent mitochondrial and genomic assays enables reliable determination of the ratio of mitochondrial to genomic DNA.
This presentation will discuss the importance of quantifying the ratio of mitochondrial to genomic DNA. Comparison between DNA derived from cell lines and that derived from three populations within the United States (African American, Caucasian, Hispanic) were examined. Initial experiments suggest that the mitochondrial to genomic quantification ratio of DNA derived from cell lines is about three times greater than DNA derived from individuals (i.e. blood).

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213. FORENSIC CASEWORK APPLICATIONS OF INNOQUANT AND INNOTYPER 21

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Retrotransposon element (RE) based systems have recently become available to the forensic genetics community. The application of these systems including analysis of actual casework property crime samples and highly degraded low level DNA samples will be presented.
InnoQuant® is a highly sensitive qPCR assay containing two high copy (>1500 copies) autosomal RE targets (80 and 207 bp) that provides comprehensive DNA quantity/degradation assessment, including an IPC for indication of PCR inhibitors and an optional multi-copy Y chromosome target. A study of 215 casework property crime samples demonstrates the utility of InnoQuant as a tool to improve laboratory efficiency. The results clearly indicate the correlation of the 207 bp SVA genetic target with the downstream STR allele recovery.
The InnoTyper® 21 kit is a small amplicon (60-125 bp) DNA typing system with 20 *Alu* markers and Amelogenin. The assay is highly sensitive for extremely degraded and/or low-level forensic samples. The application of the InnoTyper 21 system to analysis of 60 rootless hair shafts will be presented. A second study will also be presented where InnoTyper 21 profiling of excavated human remains was performed to obtain informative profiles from samples that had yielded minimal results using STR and/or mtDNA typing systems. Both studies demonstrate the utility of the InnoTyper 21 in obtaining nuclear DNA results with high power of discrimination from extremely challenging samples.
In conclusion, RE based assays have proven to be a valuable tool in a forensic DNA casework

laboratory

214. IDENTIFICATION OF MICROHAPLOTYPE LOCI FROM CHROMOSOME 22

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A microhaplotype is a short segment of DNA contains two or more SNPs. Unlike single SNPs as independent genetic marker typically ignoring the phase of the DNA, microhaplotype considerate the diploid nature of the human genome for each specific haplotype constitutes an allele. Our previous results of a genetic investigation indicated that a microhaplotype contains much more polymorphic information than divided SNPs per locus [1], microhaplotype could also be used as ancestry informative maker. This prompted us to appraise microhaplotype loci from the best sets. We report a whole genome screening results using our own software filtered the phased data from the 1000 Genomes Project. We are still improving the software. To date, 18394 candidate microhaplotype loci in chromosome 22 were picked out comprised of 65536 common frequency SNPs (MAF>0.05). The candidate microhaplotype loci were further sorted by different index, such as the number of SNPs, length of sequences and frequency of haplotypes. As a result, 2405 candidate microhaplotype loci have more than three alleles in a range of length from 3 to 199bp. We will further evaluate the loci's personal identification ability.

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215. INFLUENCE OF METAL IONS AND ORGANIC SOLVENTS BY USING LATENT FINGERPRINTS DEVELOPING REAGENTS ON DNA IDENTIFICATION

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To enhancement of detection and DNA recovery rate (%) in development of latent fingerprints, the results for the influences of various reagents based on the investigated of literature researches and some experiments were discussed quantitatively as followings. DNA molecules were commonly destructed by hydrated metal ions in the condition of protic solvents. Therefore, to protect a DNA molecules, new development method of latent fingerprints was proposed. That is, after the demetalisation agent in first step of developing experiment was treated to deposited latent fingerprints, the formated complexes from the reaction between the developing reagents and metal ions in deposited fingerprints shall be able to protect the DNA molecules from hydrated metal ion. And in case of using inorganic salts (ZnCl2) as a means of improving development of latent fingerprints in ninhydrin and their

analogues (DFO and IND) methods, the detection rate (%) of DNA molecule indicated significantly a high level in nonprotic ether solvent. Therefore, to protect DNA molecule from the hydrated metal ions, the nonprotic ether solvent condition should be used, because the formation possibility of metal (M) complex from nonprotic solvent rather than protic solvent. Thus the define of "Dual fingerprint reagent" should be modified that it can improve fingerprint detections by metal complexes and protect DNA molecule structure in a single step.

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216. INTER-LABORATORY STUDY OF A NEW IMMUNOMAGNETIC METHOD FOR SPERMATOZOA SEPARATION FROM SEXUAL-ASSAULT SAMPLES

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Analysis of sexual assault samples generally requires the physical separation of the alleged offender's spermatozoa from the victim's supernumerary amount of epithelial cells. In order to be able to handle several samples at the same time, and with automation in mind, we developed an immunomagnetic separation method (1). The final goal being to bring out a useful/informative alleged offender's DNA profile in a cost and time effective manner.
Here, we present the results from an international study, involving 13 laboratories from five different countries, using PSA-positive samples collected after sexual assaults. This study aimed at both the validation of our immunomagnetic separation method as well as comparing its separation efficiency with the different currently validated separation methods used by each of the participating laboratories.
Each laboratory was asked to handle 5 samples originating from different cases. Each sample was cut in two parts: one half was processed with our immunomagnetic separation method while the other half was dealt with each laboratory's current validated separation protocol. All laboratories were also asked to fill out a questionnaire with the time required to perform each separation, the buffers, volumes and conditions in which the extracted DNA were stored. The extracts were then returned to our laboratory where quantifications and DNA profiles were carried out. Detailed results of this study will be presented and discussed.

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217. LOW-COUNT, PURE, DIGITALLY-ISOLATED CELLS AS INPUT FOR STANDARD ANALYSIS IN FORENSIC GENETICS

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Forensic biological mixtures pose growing issues with genotype assignment for identification purposes. Today, the state-of-the-art for complex DNA profiles analysis is statistical assessment. DEPArray™ technology, a digital cell sorter with single-cell resolution, has been reported to resolve forensic mixtures through the precise separation of pure cells from admixed biological fluids.
Here we characterize the performance of standard forensic analysis methods on 100%-pure DEPArray™-isolated cells.
Aliquots of semen, saliva and blood from multiple donors (n=6), were adsorbed on swabs; cells were then reconstituted in suspension and stained with cell-specific markers. Groups of exactly 10 (WBCs n=3, ECs n=3, SCs n=2) or 20 cells (WBCs n=4, ECs n=4, SCs n=3) were isolated using DEPArray™ system, and lysed with a single-tube method. The 10 cells-pools were amplified directly with AmpFISTR®NGM SElect; the 20-cells recoveries were divided into two twin aliquots for genotyping replicates or genotyping and quantitation, along with correspondent gDNA serial dilutions for each donor.
Additionally, from each fluid, recoveries of 5, 10, 20, 41 and 83 cells were quantified using the Plexor® HY System, along with equivalent gDNA dilutions.
On average, genotyping of 10-cells recoveries showed 93.8% profile completeness and 97.9% concordance; twin aliquots obtained from the 20-cells pools showed 94.6% and 99.4%, respectively.
For both genotyping and quantitation, no statistically significant differences were found between gDNA dilutions and the corresponding cell pools.
Standards for robustness and reproducibility required by forensic genetics are guaranteed by DEPArray™-isolated cells, providing highly complete and concordant profiles from as little as 10 pure cells.

218. MOLECULAR AUTOPSY AND NEXT GENERATION SEQUENCING TESTING STRATEGIES IN SUD DEN UNEXPLAINED CARDIAC DEATHS

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Up to 30% of sudden cardiac deaths (SCD) young adults is due to sudden unexpected deaths (SUD) related to a primary arrhythmogenic disease. Recent cutting-edge next generation sequencing (NGS) technologies seem to have a key role in SUD. This study aims to raise an awareness in molecular autopsy and reveal the importance of NGS in SUD cases.
Identification of the primary cause of SCD is a critical step to define whether there is an underlying genetic etiology. It is also important to determine the impact of it on the clinical and genetic assessment of surviving family members at risk. The current molecular testing strategies include four specific genes that account for up to 35% of SUD cases. The remaining 65% remain unexplained by current strategies and suggests that some other unknown genes are also responsible. This indicates

that the developments in NGS technologies including the cardio-gene chips, whole genome and exome sequencing approaches will provide the rapid analysis of large panels of genes in a short period of time.
It is obvious that laboratories with an experience of next generation sequencing, a family-based management of the inheritable arrhythmogenic syndromes and cardiomyopathies is required for defining the guidelines for molecular autopsy and organizing a workflow from the autopsy room to the diagnotic process. To aid in the determination of the cause of such deaths, a new, validated, cost-effective molecular genetic autopsy tool and clinical screening strategy for the mutation detection in arrhythmic SCD through second generation DNA sequecing should be developed.

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219. NON CPG ISLANDS METHYLATION MARKERS: A BETTER CHOICE FOR DISCRIMINATING MONOZYGOTIC TWINS

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The DNA sequences of monozygotic twins (MZT) are highly similar. The traditional genetic markers used in forensic genetics could not discriminate them from each other. As a result, some cases involved with MZT could not be resolved. In the previous study, we utilized methylated DNA immunoprecipitation (MeDIP) sequencing technology to perform a genome-wide scan for five pairs of concordant MZT, and found a mass of DMRs (different methylation regions, DMRs) within MZT. From these DMRs, we selected four CpG islands (CGI) sequences and four non-CGI sequences located in intergenic regions to evaluate the discrimination power of them using 100 pairs of MZT. The methylation values were detected by bisulfite conversion and pyrosequencing. The mean DNA methylation levels of four CGI sequences and four non-CGI sequences were 21.37%, 0.41%, 5.05%, 1.56%, 88.18%, 87.07%, 77.45%, 87.87% respectively. 18 pairs of MZT could be distinguished by four CGI sequences in the total of 76 pairs of MZT analyzed, indicating the discrimination power of the four CGI regions reached to 23.68%. While the DNA methylation values of four non-CGI sequences showed significant differences within 81 MZT pairs out of 100 pairs of detected MZT, indicating the discrimination power was 81.00%, which was significantly higher than that of CGI sequences. Therefore, when using DNA methylation to discriminate MZT, non-CGI sequences would be a better choice compared with CGI sequences.

220. PURIFICATION AND CONCENTRATION OF DNA USING L-FUCOSE-SPECIFIC LECTIN

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DNA extraction is a very important step in DNA testing. However, crime scene samples are often of insufficient quality and quantity for DNA testing. In addition, contamination or mixed cell samples cause further difficulties for DNA testing and interpretation. Therefore, we previously developed a selective extraction method using cell-specific antibodies. In this study, we developed an alternative method using lectin, a biological material with antibody-like effects. Lectins bind specifically and reversibly with monosaccharides and polysaccharides, and are classified into a small number of specificity groups according to their binding affinity to carbohydrate polymers. By linking to each other, lectins also result in cell agglutination. Lectins were thought to have specific affinities for other types of organic matter. However, surprisingly, we found that L-fucose lectin seems to combine with 5-carboxy-deoxyribose (pentose) instead of L-fucose (hexose). Based on this finding, we successfully purified and concentrated DNA using L-fucose lectin, which can cause agglutination of DNA from historic (>6 months) bloodstains. Purified DNA and mixed cell samples were treated with lectin from *Ulex europaeus*, and then boiled at 98°C for 10 min to precipitate the lysate. This supernatant contained the DNA, which was used as template for PCR analysis and DNA typing. After comparing it with non-lectin-treated DNA, DNA treated with lectin showed enhanced levels of amplification, with less background. This result indicates that lectin treatment improves the purity of extracted DNA, as the binding of L-fucose-specific lectin to deoxyribose allows the specific collection of DNA from the sediment of lectin agglutinations.

221. RAPIDHIT FOR THE PURPOSE OF STAIN ANALYSES – AN INTERRUPTED IMPLEMENTATION

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Rapid DNA instruments have in recent years been developed, enabling analysis of forensic samples with a minimum of human intervention. Initially intended for fast handling of reference samples, such as samples from suspects in booking suites, attention was broadened to include crime scene samples. The aim of this study was to determine whether or not the RapidHIT System (IntegenX) is fit for crime scene samples. The first runs gave very poor results, which was found to be due to an incorrect firmware setting leading to no or just minute amounts of amplicons being injected for electrophoresis. After solving this problem, 28 full runs (seven samples each) applying NGM SElect Express were performed comprising various amounts of blood on cotton swabs. Six of the runs failed completely, four due to cartridge leakage and in two runs the PCR mix was not injected. For 155 samples with 1-5 µL blood (volumes for which complete DNA profiles are expected), 119 samples (77 %) gave complete DNA profiles. Among the most serious failures were incorrect allele calling and leakage of DNA extract or PCR product. Other general issues were failure to export results, anode motor breakdown and broken capillary array. Due to the encountered problems with software, hardware and cartridges, together with the low success rate, it was decided not to continue towards implementation of the RapidHIT System in casework.

222. SPERM DNA CAPTURE: A NOVEL APPROACH FOR SEPARATION OF NUCLEIC ACIDS IN MIXED SEXUAL ASSAULT SAMPLES

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Analysis of DNA from sexual assault samples presents unique challenges due to the presence of victim DNA, sample degradation, and trace DNA from the assailant. Conventional analysis of these samples relies on Differential Extraction that is laborious and requires intact sperm heads to allow separation of the two DNA fractions. We've developed a novel approach for isolation of sperm DNA from mixed samples based on affinity capture of proteins associated with sperm DNA. Chromatin-based sperm DNA capture takes advantage of the unique form of chromatin found in sperm cells. The DNA binding proteins protamine 1 (PRM1) and protamine 2 (PRM2) replace most histones during the haploid phase of spermatogenesis. We have modified techniques used in traditional chromatin immunoprecipitation to target the DNA/protamine complex from solutions derived from cell mixtures. The sensitivity of capture was routinely demonstrated on as little as 100 input sperm. Results demonstrate the feasibility of the method which incorporates rapid sample lysis, novel antibody incubations conditions, and simplified sample purification in a workflow compatible with STR analysis. Chromatin-based sperm DNA capture has the potential to enable successful processing of sexual assault samples that are classically refractive to analysis, such as aged/lysed samples. Sperm DNA Capture has eliminated many of the manual steps inherent in Differential Extraction, and can be fully automated. Results suggest Sperm DNA Capture is a more highly sensitive and specific method for separation of sperm DNA from a mixture than Differential Extraction. Method development was supported under NIJ award 2014-DN-BX-K017.

223. THE ADVANCES OF FORENSIC DNA TECHNIQUES IN MALAYSIA

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Forensic DNA analysis has gone through tremendous advancement at every process involved. From the hands on extraction via organic or Chelex, it has progressed to automated platform via Solid Phase Extraction (SPE) methods. Nowadays the SPE is bundled in cartridges and all that needs to be done, is to cut the samples with minimal pre-treatment and in the latest technique available; the extraction step is totally eliminated. The next process, quantitation is coupled with extraction to ensure the amount of DNA extracted and even so it has develop to not only detect male DNA but also the rate of degradation. Whereas in the amplification process, the number of locus analysed for individualization has improved to accommodate the latest FBI recommendation which is to increase the minimal number loci analysed to 20 core loci all in a single multiplex.
The latest innovative technology is the elimination of extraction, quantitation and amplification process;

i.e the crime sample is placed into the cartridge and in 1 hour, the DNA profile at 24 loci is generated. The Next Gen Sequencing (NGS) technique also emerged with the ability to generate results for >150 loci in a single multiplex which promised the ability to determine not only the colour of the eye and hair but also the ancestry of an individual. All the latest technique implemented is useless if the results generated are not deciphered by trained DNA analyst with all the quality assurances in placed.

224. THE EFFECT OF ENZYME DIGESTION TIME ON THE DETECTION OF DIATOM SPECIES

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This study is aimed at detecting diatom in lung, liver and kidney tissues using PCR - DHPLC technology after different periods of enzyme digestion to assess the effect of enzyme digestion on the detection of diatom species. Twenty Randomly selected experimental rabbits were drowned at the same place. Their liver, kidney, and lung tissues were removed for sampling. After the extraction of DNA from the samples, amplification was conducted with specific primers of the SSU gene of diatom. Then, an analysis was performed with agarose gel electrophoresis and DHPLC. Within 2 h-8 h, the amount of the diatom species found in the lung gradually increased over time and was statistically significant ($P<0.05$). After 8 h, with enzyme digestion, the amount of the diatom species found in lung showed no significant increase ($P>0.05$). However, as for the liver and kidney, within 2h-6h, the amount of the diatom species gradually increased over time and was statistically significant ($P<0.05$). After 6h, the fig. did not present significant growth ($P>0.05$). The amount of the diatom species found in the organs after different periods of digestion time had significant differences, which provides a reference for the detection of diatoms and also, has a good application prospect in the forensic identification of drowning. PCR-DHPLC method can separate and identify different diatom species, it can compare the diatom species in drowning organs to drowning waters to determine whether the diatom species are the same and thus it provides a better way to infer the drowning locations.

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225. THE PRESERVATION OF DNA FROM SALIVA SAMPLES IN SUBOPTIMAL CONDITIONS

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Population genetics requires a range of DNA samples from several population groups. This means that samples may need to be collected from donors inhabiting remote and isolated locations. These remote locations may not have optimal storage facilities to preserve the collected biological material. In these cases saliva has been established as a good alternative source for genomic DNA as it can be stored at room temperature [1].
In this project an economical saliva collection method was optimised to compete with existing saliva collection kits with similar preservation results. Commercial kits such as Oragene•DISCOVER (OGR-500) and Norgen Saliva DNA collection and preservation device are able to preserve saliva samples up to 5 years[2-3]. Once collected, the saliva was stored in a dark environment without refrigeration.
The extracted DNA was quantified using a qPCR method and genotyped using AmpFlSTR® Identifiler® Plus. The DNA concentrations obtained from 250µl saliva + 250µl buffer ranged between 10 and 40ng/µl. The integrity and quantity of the samples were obtained for all time periods up to two years. Additionally; full profiles were obtained for all tested samples for all time periods up to two years. In conclusion, the novel storage buffer was shown to preserve the genomic DNA over this period. Full profiles were obtained after the two year period at room temperature in a dark environment.

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226. Y-SCREENING AND DIRECT AMPLIFICATION OF CASEWORK SAMPLES

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Current serological screening of sexual assault samples is time consuming and often shows mixed success. Because the newest qPCR and STR amplification chemistries demonstrate robust tolerance of PCR inhibitors, it is possible to perform direct amplification from casework samples and eliminate the DNA purification step. Doing so streamlines the workflow and reduces cost and improves yields, especially with low level DNA samples. The Casework Direct Kit enables the rapid generation of lysates from casework samples, which may then be used in amplification based assays such as the PowerQuant® System to quantify human DNA, determine the male/female DNA ratio, predict PCR inhibition, and assess DNA degradation. If preferred, the lysate can be used directly in an STR amplification assay to generate an STR profile. In the case of sexual assault samples, the lysates are used in the PowerQuant® System to screen for the presence of male DNA. Based on this information, the analyst can decide to perform differential extraction on the sample, take the lysate directly into an STR amplification reaction, or to stop processing the sample. Quantification results from the lysate can be used to normalize template DNA in downstream STR amplification reactions. Processing samples with the Casework Direct Kit facilitates improved results by directing workflow decisions and minimizing

repeat assays and/or sampling. We will present data from Y-screening and mock casework applications such touch DNA samples, including the ability to detect the presence of male DNA and a full PowerPlex® Y23 System profile in a 96 hour post-coital sample.

227. "INHIBITING INHIBITORS": PRELIMINARY RESULTS OF A NEW "DNA EXTRACTION-AMPLIFICATION" DISINHIBITION TECHNIQUE IN CRITICAL HUMAN SAMPLES

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The present study focused on the genetic analysis of a presumptive Bronze Age family burial, with at least six skeletons (Plana Castell, Barcelona, Spain).
In order to investigate the individuals' biological affiliation, a molecular study was undertaken, studying three bone samples of each of the three physically best-preserved individuals. After four amplification attempts, it was impossible to obtain a positive DNA amplification. The excavation data indicated that the skeletons were buried in an old location used as a dumping ground, being very likely the presence of biological and/or chemical contaminants. To overcome this difficulty, we undertook several modifications both in the extraction as in the amplification protocol (1). For example, during different protocol phases, we tested distinct incubation times, $MgCl_2$ concentration, the inclusion of Bovine Serum Albumin (BSA) in the amplification protocol (2), and different buffers with distinct pH. A correct acidity or alkalinity range will favour a stronger junction of the contaminant to a certain buffer structures, allowing its removal during the process (3).
Despite their antiquity, the preliminary results indicate that all samples were effectively inhibited, and for the first time we were able to obtain mtDNA results, specifically, for the Hipervariable mitochondrial region 1. It also allowed us to infer that our protocol performance increased considerable, mainly the amplification modifications. In general, the best results were obtained with $MgCl_2$ 0,6 µL (20mM) and BSA 2mg/mL per sample. Our result is quite important in a forensic analysis, mainly in cases where corpse/s is/are buried directly in the soil.

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228. A COMPREHENSIVE STUDY ON THREE QPCR QUANTIFICATION SYSTEMS AND THEIR INTERPLAYS WITH DIFFERENT PROFILING SYSTEMS

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DNA retrieved at crime scenes are far from pristine and very often subjected to various degree of degradation and/or inhibition. In order for the forensic scientists to formulate the best strategy to tackle the challenging DNA samples in a timely and efficient manner, it is in particular advantageous if one can obtain such information at an early stage of DNA analysis, ideally during DNA quantification. DNA quantification plays at least three important roles in the overall DNA profiling process. First, it assesses both the efficiency and purity of the DNA extraction process. Second, it offers an estimation of how much DNA template to be added into the subsequent DNA profiling analysis to ensure optimal amplification of DNA target. Third, it allows an estimation of DNA degradation in the samples. Here, we evaluated the performance of three selected qPCR kits, namely the Investigator Quantiplex HYres Kit from QIAGEN, the Quantifiler Trio DNA Quantification Kit from Applied Biosystems™, and the PowerQuant® System from Promega. We first compared the precisions and consistencies of these three selected qPCR kits. We also examined their performances in the detection of a trace amount of male DNA in a mixture, and detection of common inhibitors in mock samples. Finally, we studied their interplays with several commonly used STR profiling systems in an attempt to understand their combining performance. We believe that our work can offer useful information for laboratories to select the best combination of quantification and amplification kits according to their requirements and operation needs.

229. A MULTIPLEX SNAPSHOT ASSAY FOR NON-INVASIVE PRENATAL PATERNITY TESTING FROM MATERNAL PLASMA: A PILOT STUDY

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The discovery of cell-free fetal DNA (cffDNA) in the maternal plasma has opened up new approaches for non-invasive prenatal paternity testing. However, the amplification of fetal autosomal alleles from maternal plasma is not sufficient for reliable paternity testing due to heavily suppressed by maternal DNA. Previous studies have demonstrated that several genomic DNA regions are hypermethylated in the placenta and hypomethylated in maternal blood cells. This methylation pattern allows the use of methylation-sensitive restriction enzyme (MSRE) digestion for detecting placental-derived hypermethylated cffDNA in maternal plasma. The aim of this study was to develop a SNaPshot assay based on MSRE digestion PCR (MSRE-PCR) to determine fetal single nucleotide polymorphism (SNP) genotype. Five regions from *SKI*, *PAX3*, *TLX3*, *PSMB8*, and *CHST11* gene, containing 2~6 placental hypermethylated CpG sites overlapped with *Hpa*II or *Hin*P1 I recognizing sites and 1~2 SNPs within a short amplicon were selected for developing the SNaPshot assay to detect 8 SNPs. The *ACTB* gene was chosen as a control to confirm complete enzyme digestion. SNP genotypes were analyzed in plasma of 11 1st-trimester, 10 2nd-trimester, and 14 3rd-trimester pregnant women. After enzyme

digestion, hypomethylated *ACTB* sequences were not detectable in all 35 pregnant women, and SNP genotypes of plasma DNA was identical to the fetal genotype in each case. We concluded that fetal DNA detection from maternal plasma is a promising technique but more methylation markers are needed for non-invasive prenatal paternity testing. Alternatively, universal fetal marker included in the multiplex assay can be applied to prenatal diagnostic.

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230. A NEW MULTIPLEX ASSAY OF 20 INDEL LOCUS FOR FORENSIC APPLICATION

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Insertion-deletion (InDel) markers can be considered as an important complementary tool for common STR markers in human identification because of the advantages such as low mutation rates, no stutter, and potential small amplicon sizes. This study described a multiplex assay of 20 InDel loci identified by the fluorescence amplification and the capillary electrophoresis detection technology. These loci, which belongs to biallelic Indels, were all distributed across autosomes except two which located at X and Y chromosomes, respectively. The nucleotide number of InDel spanned from 2 bp to 7 bp, and the values of minimum allele frequency were between 0.20 and 0.50 in major population groups. All amplicons were designed in the size ranging from 97 to 250 base pairs to satisfy the degraded case samples. We assessed these InDels from sensitivity, precision and accuracy, species specificity, tissues homologous, and degraded sample analysis. The sensitivity of the system could detect as little as 0.3 ng DNA template. In addition, the panel had a high rate in analyzing the degraded DNA. Our results displayed that the multiplex InDel assay is a reproducible, accurate, sensitive, and robust tool for forensic investigation.

231. A NEW STRATEGY FOR A "DIRECT" AMPLIFICATION OF FORENSIC SAMPLES

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One of the most effective ways for human identification is the genetic analysis of the crime scene biological evidence(s). However, such studies could be expensive, and it could take too long until the genetic profile achievement (1).
In the present work we performed modifications to the conventional extraction DNA protocol (1), in order to perform a "direct" amplification, trying to obtain the optimal conditions for generating a fast and reliable genetic profile. To perform so, we employed different fresh blood and saliva samples, and carried out an extraction with the Prep-N-Go™ buffer (ThermoFisher™ SCIENTIFIC, Foster City, USA) during a 30 second incubation. After another 30 seconds centrifuging, the extract was collected to perform the autosomal amplification with GlobalFiler™ Express PCR Amplification Kit (ThermoFisher™ SCIENTIFIC, Foster City, USA), with three different volumes (1µL, 2µL and 3µL). The quality of the new procedure always took into account the minimum standards of quality, estimated by the allele's height in RFUs. On the other hand, it was also evaluated if our new proposed protocol maintains the required quality, performing sensibility, reproducibility and contamination tests.
We were able to conclude that it was possible to obtain a reliable genetic profile performing a direct amplification, although the best amplification conditions varied according to the type of sample. In general, the best obtained results were determined in a range of 1µL-2µL extract volume, and it was possible to obtain a genetic profile in 90 minutes.

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232. A PERFORMANCE STUDY ON THREE NEW GENERATION 6-DYE DNA PROFILING SYSTEMS

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The Federal Bureau of Investigation (FBI) has recently expanded the CODIS core loci from the existing 13 to 20 as a new guideline, and laboratories from the US are required to comply with the new regulations before uploading or conducting identity search in the national database. The expanded CODIS format, which shares all the core loci commonly used in the European countries and the US, not only increase international compatibility, but also reduce the number of adventitious matches, and hence facilitates international law enforcement and counterterrorism endeavours. Here, we report the effectiveness and performance of three new STR amplification systems with 6-dye chemistry, namely the Investigator 24plex QS Kit from QIAGEN, the GlobalFiler™ PCR Amplification Kit from Applied Biosystems™, and the PowerPlex® Fusion 6C System from Promega, with respect to: average peak height, detection sensitivity, heterozygous peak height balance, intra-colour signal balance, capability of male detection in mixed DNA, consistency of the preservation of mixture ratio, and tolerance to common inhibitors. Our work has demonstrated that these three profiling systems have their specialties, and hence it is recommended that laboratories should select the most suitable kits according to their own requirements and operational needs.

233. ACCURATE QUANTIFICATION OF FORENSIC SAMPLES

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In the workflow of the DNA typing the quantification plays a key role, especially when very few human cells have to be analyzed. Technical challenges associated with STR profiling of low amounts of DNA, have been resolved with the introduction of the latest generation kits that enables forensic laboratories to simultaneously obtain a quantitative and qualitative assessment of total human DNA. Quantifiler® Trio DNA quantification kit was validated in our laboratory according the guidelines approved by the ENFSI and extensively used before STR amplification of forensic casework DNA samples. The maximum reproducibility of the analytical system is demonstrated in the range between 500 and 5 pg/µl, and therefore is particularly suitable for the quantification of very small traces. When the DNA quantity exceeds this value, the inhibitory effect increases. We have extensively used this system on various types of forensic samples, including mixed samples taken directly from the corpse. Extremely interesting is the actual correlation of the negative results of the quantification by the absence of genetic profiles, as a result of DNA typing. So, this analytical system can also be used for the reliable identification of human origin of a forensic sample. Here, we show the results obtained from the validation process in our laboratory.

234. AMPLIFICATION OF DEGRADED DNA: THE GENOTYPING POTENTIAL OF 32 X-INDELS MARKERS IN BONE SAMPLES

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The genotyping success of degraded samples, such as skeletal remains, is influenced by DNA concentration and fragment lengths that can be amplified. Thus, techniques used in human identification that generates reduced amplicons size (50-150 base pairs-bp) tend to be more successful in DNA amplification. The aim of this study was to evaluate the amplification efficiency of 32 X-InDels markers in DNA extracted from human bones. Five samples were selected (4 femurs and 1 tooth) of individuals who died between 5 and 13 years ago, with 54 to 89 years old at the time of death. Bones were macerated and DNA extraction was performed using extraction columns. PCR amplification was performed in multiplex for the 32 X-InDels markers. Until yet, it was not possible to amplify any marker of DNA extracted from tooth. For one femur sample it was not possible to identify only the genotype of the MID3719 marker; for the other three samples it was not possible to identify the genotype of the MID3719 and MID2089 markers. In the analysis of positive control made with blood sample we can observe that the MID3719 and MID2089 markers commonly present low peaks (in RFUs), therefore the difficulty to amplify these markers in degraded samples was an expected phenomenon. The age and time of death appear to have no influence on DNA amplification of these markers. These data suggest that 32 X-InDels may actually be efficient in amplifying degraded samples, especially because all the amplicons in that panel have less than 150bp.

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235. AN INVESTIGATION OF TEN DIP-STR MARKERS FOR UNBALANCED DNA MIXTURES AMONG SOUTHWEST CHINESE HAN POPULATION

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In forensic routine works, unambiguously genotyping DNA mixtures is still a tough problem which remains unsolved. Although the method based on STR markers and related calculation software has been developed, it still has a limitation in detecting extremely unbalanced mixtures with a ratio larger than 10:1. DIP-STR, a new method introduced by Hall, D. et al(1), performed well in discriminating the minor DNA from 1000-fold major DNA mixture. In this study, we selected 10 DIP-STR markers performing well in Swiss population to investigate the potential usefulness in southwest Chinese Han population. Allele frequencies were estimated based on 52 samples and probability of informative markers (I value) was calculated to evaluate the usefulness of these markers in detecting DNA mixtures. Six of the ten DIP-STR makers with a relatively high I value (above 0.25) indicated the usefulness in detecting mixtures in southwest Chinese Han population. All the markers except for one could detect the minor DNA at 1:1000 scale. However, the corresponding personal identification power was far from enough using these six markers. Therefore, we suggest a panel with more loci is imperative and a panel combined with DIP-STR and SNP-STR markers(2) may be a possible way to achieve it.

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236. ANALYSIS METHOD OF Y-STR MUTATION INTO FAMILIAL SEARCHING

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The new-established method 'Y-STRs familial searching', built upon the Y-STR test, has already becomes a powerful tool for criminal detection. However, Because of the Y-STRs mutateions frequently during their descending course, between father and son, resulting in the discrepant Y-STR genotypes to be emerged in the consanguineous male relatives of same kin. The more distantly related men are, the higher the chance that they will have distantly Y-STR haplotypes. Therefore, even if there are similarity of Y-STRs genotyping were similarity between the evidence at the scence and certain

individualsuspected samples screened, leading to the difficulty is still at presence to judge whether the matched man's family is really include the suspect's. Here, the analytic strategy and principle is illuminated about the data from familial searching based on aA typical case that which exist four mutations of four Y-STRs siteswere found in pedigree screening, is cited in the article to illuminate data analysis strategy in familial searching.

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237. APPLICATION OF DNA PROFILING IN EVIDENCE EXPOSED TO MARINE ENVIRONMENT

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DNA profiling from evidences left at the scenes of incidents/accidents has been used as an important tool in solving cases. Bloodstains and body fluids remaining in crime tools demonstrate criminal facts by proving to whom they belong even after a long time through DNA Profiling. However, in most capital cases, criminals abandon the evidence in the water or woods to hide their acts, and increasingly more evidence become damaged by environmental factors.
Especially, scientific analysis techniques are needed since it is difficult to secure underwater evidence from a marine incident/accident and very likely to get damaged, affected by waves, water temperature difference, ocean currents and marine organisms.
In this study, we posit the possible existence of investigative clue in crime tools or lost items recovered from the sea and examine the impacts of changes in underwater evidence, the environment and the properties of the specimens on DNA analysis.
This experiment classified evidence typically found at crime scenes according to material, dropped blood, dipped them in sea water, detected the DNA of micro-traces (latent bloodstains) of evidence and analyzed their genotypes. DNA recovery rate of specimens of a small amount of bloodstain evidence was low, damaged and decomposed by various microorganisms as exposed to harsh conditions for a long time; however, we could obtain DNA profiles even from 10-day-old evidence through STR-analysis. Thus, this study establishes a method to increase the evidence detection rate in marine environment and suggests utilization as objective data in securing forensic genetics information on marine incidents/accidents.

238. APPLICATIONS OF ARBITRARY-PRIMED PCR TO VEHICLE DETRITUS

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The interior and exterior of vehicles comprise an extremely difficult field with targeted primers. This poster examines the utility of arbitrarily-primed amplification with untargeted primers and NGS technology.
When facing a mass of sequences from some real source such as a vehicle there are questions to be asked of the DNA-sequences roughly, in the below rank-order:
Coherence* applies to the internal coherence, a property or characteristic of metagenomes, where the relative ratios of the component organisms is, to some degree, fixed with respect to each other.

1. How many entities are involved? For examples, how many vertebrate species are present, if any. How many coherent*-metagenomes, if any? Is there a non-coherent* suite of soil-communities?
2. What can possibly be inferred about the components provenance(s)?
3. Does the mixture being examined have components that can be reasonably inferred as matching specific items like a soil at a particular locality, a vertebrate species like chicken or human.
4. In any particular case, do the results from this untargeted technology indicate that it may be advisable or worthwhile to shift over to conventional targeted technology using specific primers for specific species?

239. CAPTURING SPERMATOZOA FOR STR ANALYSIS OF SEXUAL ASSAULT CASES USING ANTI-SPERM ANTIBODIES

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DNA isolation in sexual assault cases is complicated by the presence of large numbers of female epithelial cells, which are often in vast excess when compared to spermatozoa. The two-step differential extraction has become the standard method for isolating the spermatozoa; however, new techniques in forensic science allow the use of precision techniques for capturing spermatozoa. In an attempt to refine the process we have used an immuno-magnetic bead-based technique for sperm cell separation. We identified two antibodies that were specific to spermatozoa: SP17 polyclonal antibody and SP10 Intra Acrosomal Protein monoclonal antibody. These were conjugated to Dynabeads® M-450 Epoxy beads. We used these antibody conjugates to isolate the spermatozoa in samples that exhibited the characteristic of sexual assault samples. Microscopy showed the successful separation of spermatozoa in the samples with sperm concentration 10^4/ml and 10^3/ml and STR analysis produced full male profiles, similar to the result of the samples extracted using the two-step differential method.
Mixed profiles were seen for the samples with 10^2/ml sperms and incomplete male profiles with female STR peaks as major contributor when the density of the samples were 10^1/ml in comparison to the established two-step differential method. As a result, our finding suggested the possibility to isolate the spermatozoa of sexual assault samples using magnetic beads coupled to antibody against sperm specific proteins and it could be available for application in routine work as an alternative to conventional methods.

240. CLASSICAL APPROACHES TO ANALYZE THE MICROHAPLOTYPE

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Previously, we have introduced approaches of single chain sequencing based massively parallel sequencing technology (MiSeq) and PCR based Single Strand Conformational Polymorphism technology (SSCP) to directly genotype the microhaplotype [1]. In the present study, several other classical technologies, which have the ability to detect sequence variations, were performed to attempt to analyze the microhaplotype. The technologies including denaturing gradient gel electrophoresis (DGGE) and high resolution melt (HRM). DGGE is a form of electrophoresis which use chemical gradient to denature the sample as it moves across an acrylamide gel. HRM analysis is to monitor the precise warming of the amplicon DNA. The melting temperature shaped melt curve of the amplicon is dependent on the sequence of the DNA bases. Our results indicated that the two methods both lack enough resolution power to separate every different allele of a microhaplotype locus. However, they could still distinguish part of the alleles under appropriate conditions. Considering the convenience and high cost performance of DGGE and HRM. They could be used as complementary methods to genotype the microhaplotype combined with sanger sequencing.

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241. COMPARATIVE ANALYSIS OF IRFILING AND IDENTIFILER HUMAN IDENTIFICATION KITS IN IRANIAN POPULATION REVEALS ALLELE DROPOUT

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In modern forensics, scientists use13 to 24 short tandem repeats (STRs) markers for forensic investigations, paternity indexing, etc. To create unique DNA profiles for different purposes. IRFiling kit from Kawsar Biotech Co. (KBC, Iran) shares 15 STR loci with AmpFISTR Identifiler kit (ThermoFisher, USA), namely D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, FGA, D5S818 and the sex typing Amelogenin. In addition the IRFiling has SE33 STR marker which has been shown to have high heterozygosity in different populations. This locus is used in NGM Select and GlobalFiler kits as well. IRFiling kit uses different sizes and dyes compared with the Identifiler kit. To compare and validate the performance the IRFiling a parallel study was carried out.
We simultaneously compared the alleles generated by AmpFISTR Identifiler kit (ThermoFisher, USA) and the IRFiling kit.
More than 300 DNA samples from Iranians were used in parallel to compare the results obtained. For both kits the multiplexing was done according to the manufacturers' recommendations.
Our result showed that out of 300 samples the Identifiler showed 3% allele dropout for the D19S433 markers. allele dropout was confirmed by Sanger sequencing with D19S433 primers.For other markers no difference was observed. Both kits performed well and all samples produced similar heights

and alleles. Since then the IRFilling kit has been used on more than 3000 samples with outstanding results.
Our result showed that the IRFiling kit can be substituted for similar kits in the market with desired results.

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242. COMPARISON OF THE DNA EXTRACTION, PURIFICATION AND PROFILING TECHNIQUES IN BONE AND TEETH SAMPLES EXPOSED TO HIGH AND EXTREME TEMPERATURES

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Effective DNA extraction and amplification methods from degraded human remains are always challenging and critical in successful DNA analysis. In our study, we aimed to compare different DNA extraction and purification methods including Phenol-Chloroform-Isoamyl Alcohol, Qiaquick DNA Mini Kit, DNA IQ System ve Genorise Bone DNA Extraction Kit and HiPurABone DNA Extraction Kit, CTAB/Isoamyl Alcohol-Chloroform, Sodium Acetate, Chelex 100, Sodium Hydroxide, Amicon Ultra-4 Centrifuge Filters, Qiaquick-based Bosnian method, Sefadex 50, Invisorb Spin Forensic Kit and Genematrix Bone DNA Purification Kit in burned 25 bone and 25 teeth samples exposed to different degrees of temperature ranging from 50oC to 1000oC for different times in order to develop a new modified method as the current techniques are complicated, time-consuming and not globally standardized. We also collected 50 oral swab samples from each case to be used as control. We pulverized the samples and used 50 mg to 3 gr decalcified powdered samples to isolate DNAs.
After the isolation of DNA molecules, DNA quantities were determined with Qubit Fluorometer. The DNAs were amplified with AmpFlSTR® Identifiler™, MiniFiler™ and *GlobalFiler*™ PCR Amplification Kits, PCR products were electrophoresed on ABI 3130 Genetic Analyzer and the results were analyzed in the GeneMapper. According to our results, maximum quantity of DNA was obtained with our modified method in which Phenol-Chloroform-Isoamyl Alcohol and Qiaquick DNA Mini Kit were used together. Maximum temperatures which we obtained the DNAs were 190oC for burned bone samples and 400oC for burned teeth samples. Our genotyping results will be presented at the congress.

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243. COMPARISON OF THE EFFICIENCY OF SE33 MARKER AMPLIFICATION BETWEEN NGMDETECT AND GLOBALFILER KITS BASED ON AN ANALYSIS OF A HIGHLY DEGRADED BONE MATERIAL

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Obtaining a full STR profile when working with degraded DNA in human identification cases is one of the most challenging tasks faced by forensic genetics. Many research group lead studies trying to improve DNA profiling from degraded bone material. When it comes to the degraded DNA the amplification rate decreases with the increasing length of a marker. For example, the SE33 marker in GlobalFiler kit has up to 500bp and is the first marker to have drop-outs. Recently, a new kit was introduced, namely NGM Detect in which SE33 was shorten up to 351bp. To check it's suitability for a degraded DNA we analysed biological samples coming from the Second World War. Used remains were found in a mass grave during an exhumation which has taken place in Białystok (Poland) under the project of The Polish Genetic Database of Totalitarianisms Victims. We tested 54 tooth samples and compared the amplification rate between two mentioned kits. Degradation index in all the samples indicated that the DNA was significantly degraded and DNA concentrations were lower than 0,01 ng/μL for most of them. Comparative studies showed a difference amplification rate for all the autosomal STRs but the biggest was noticed for SE33 marker.

244. COMPARISON OF THREE COMERCIAL KITS TO THE ESTABLISHMENT OF STR GENETIC PROFILES ON CRITICAL SAMPLES

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Nuclear DNA analysis provides great and crucial information to personal identifications and to kinship analysis. Nevertheless, nuclear DNA from human skeletal remains usually shows several problems related with its scarcity (LTD or Low Template DNA) and its modifications. Such problems compromise its analysis and results interpretation [1]. For this reason, there have been developed different technics to face up to these problems, many of them based on the improvement of STR amplifying kits, for example the amplifying of miniSTRs instead of classical STRs [2].

In the last few years, there have been a development of many of these kits, mainly those ones that concern autosomal STR amplification on critical samples. Through the present study, we would like to evaluate the efficiency of three of them (AmpFℓSTR® MiniFiler™ PCR Amplification Kit, AmpFLSTR® NGM™ PCR Amplification Kit, and PowerPlex® ESX 17 System). For this purpose, there was carried out a STR analysis on 84 skeletal and/or dental samples from 42 skeletal human remains (two samples from each individual) dated back between 2000 and 5000 years of antiquity [3]. Here we discuss the obtained results evaluating the performance of each kit.

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245. COMPARISON OF TWO DIFFERENT DNA EXTRACTION METHODOLOGIES FOR CRITICAL BONE OR TEETH SAMPLES

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Through this study we compared two DNA extraction methods for skeletal or dental human samples. For such task there were analysed 38 samples from 19 individuals, selecting two samples from each one. When it was possible, we selected dental complete samples, without cracks or cavities, and also with a natural light colour. There were selected preferably skull or diaphysis of long bone.
The two samples from each individual were processed individually in two different laboratories (Laboratories 1 and 2). There were employed a different DNA extraction methodology in each laboratory, applying in Laboratory 1 the protocol proposed by Rohland and Hofreiter [1] [2] [3], and in Laboratory 2 a commercial kit for amplified products purification [4]. Finally, in order to compare the efficiency of both methodologies, in Laboratory 2, a DNA quantification by Real Time PCR (RTPCR) was performed, by the amplification of two different size mitochondrial DNA fragments [5]. In this way, it was possible to evaluate the efficiency of each protocol, and to discuss advantages and disadvantages of each one.

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246. DETECTION OF MICROSATELLITE INSTABILITY IN BREAST CANCER PATIENTS

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Short tandem repeat sequence (STR) can be found in genome. This loci are used for paternity testing and identification in forensic science since 90's. The DNA identification from references materials, which belongs to missing person, are very important in such cases; mass disasters, mass terror attacks, airplane crash etc. When there is no available relatives or reference personal items, dental records or pathology samples (paraffin embedded tissue) could be used for human identification as a reference sample. In this study, we have investigated the instability of STR markers in cancer patients those effected with breast cancer in order to evaluate the occurrence of the loss of heterozygosity (LOH) and microsatellite instability (MSI). We collected healthy and tumor tissues from 40 patients with their buccal swab samples as a control sample. We tested 15 AmpFlSTR Identifiler PCR Amplification Kit (Thermo Fisher Scientifics) loci for determining the differences between the buccal swabs and tumor tissues. There are a number of studies on microsatellite instability in different types of tumors by comparing the STR profiles of malignant and healthy tissues on the same individuals. Therefore we are expecting to detect genotype differences between breast tumor tissue and buccal swab samples of 40 participant.

247. DEVELOPING EIGHT SNP-STR MARKERS FOR DNA MIXTURE DETECTION

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SNP-STR maker is a combination of a SNP marker and a tightly linked STR marker which has been introduced to improve the discrimination power of STR markers for a long time. Recently, some researchers have reported that SNP-STR markers performed well in detecting unbalanced DNA mixtures by designing two allele-specific primers.(1) In this study, STR markers were selected from previously reported articles which had a high polymorphic diversities in Chinese Han population. Then SNP markers with MAF above 0.20 and located no more than 250bp from the STR markers were screened in SNP database. Based on these criteria, eight SNP-STR markers were finally developed and ARMS-PCR method was used to achieve allele-specific amplification which could exactly amplified the minor's DNA from an unbalanced mixture. 20 samples were used to estimate the polymorphism of

the SNPs in these eight markers. Six of the eight loci had a high polymorphism in SNP (above 0.20) which indicated a potential in genotyping DNA mixtures. The probability of successfully discriminating a mixture using these eight SNP-STR markers is 0.924932. However, it is imperative to get a larger number of these loci to improve the probability of discriminating a mixture. Therefore, further studies are necessary in finding out more SNP-STR markers.

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248. DEVELOPMENT AND VALIDATION OF 20PLEX STR PANEL

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Short Tandem Repeats (STRs) analysis is the widely used for forensic applications. In this study we aimed to develop a single reaction, fluorescently labelled in-house STR panel containing 20 different loci. We chose 18 loci from two commercial kits (AmpFlSTR® Identifiler®, Thermo Fisher Scientific, and The PowerPlex® 16 System, Promega) and combined it with two miniSTRs (D6S1043 and D12S391) which does not exist in either of the kits to constitute a 20plex panel. Primers for each loci were designed and labelled with four different dyes considering that they should not overlap in the mix. Primarily, each loci was amplified alone to check the amplification efficiency and observe its size. After the optimization of the each loci we successfully combined 20 STRs in a single multiplex. DNA concentration and primer volumes were adjusted to get an efficient and balanced amplification. Validation study of these 20 STRs were performed as following parameters and aspects: analytical threshold, sensitivity and stochastic threshold, heterozygous balance, precision and accuracy, repeatability and reproducibility, genotype concordance (9947a), DNA mixtures, and case samples. The high performance of this 20plex fluorescent PCR system makes it a valuable alternative to the current commercial kits and can be used widely for forensic purposes.

249. DEVELOPMENT AND VALIDATION OF INVESTIGATOR QUANTIPLEX PRO, A NEW ROBUST QPCR QUANTIFICATION ASSAY EXAMINING QUALITY AND INTEGRITY OF HUMAN DNA IN FORENSIC SAMPLES

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Human DNA isolated from various sources has to be assessed in terms of quantity, quality and integrity prior to STR analyses, since these are complex multiplex systems that require a defined range of input DNA and template quality to perform accurately. As DNA quantification is the only step preceding the STR PCR it is essential to extract as much information as possible from this reaction to aid correct setup of STR reactions. With the new Investigator Quantiplex Pro quantification assay we can address the amount of amplifiable DNA, the presence of inhibitors and the integrity of DNA samples in one

reaction to ensure a high correlation between quantification and STR results. The qPCR assay uses a novel PCR fast-cycling technology and provides rapid, robust and precise quantification and a high sensitivity for male DNA even in the presence of high amounts of female DNA. We will present data from our current development and validation.

250. DEVELOPMENT OF A SNAPSHOT SYSTEM FOR SNPS INVOLVED IN THE ADAPTATIVE RESPONSE TO HIGH ALTITUDE HYPOXIA

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An efficient alternative to Sanger sequencing is SNaPshot technology. SNaPshot enables multiplex amplification of several regions of interest including genes from different chromosomes. This approach is used routinely in various fields of research and several sample types like paraffin embedded material or degraded forensic samples. Our work describes a SNaPshot system for detection of six SNPs: rs1868092, rs2881504, rs13419896, rs4953354, rs895436 and rs1867785, which are located in HIF2-α gene. Primers and probes were designed using Assay Design Suite V2.0 software and verified in Primer-BLAST and UCSC & in silico-PCR websites. Primer-dimer and hairpin were analyzed using Autodimer software, PCR was 1X QIAGEN®-Multiplex-PCR-Kit, 1μM each primers, 10ng DNA, thermal profile: 95°Cx15 min, 45 cycles: at 94°C(1min)-62,7°C(1min)-72°C(1,5min) and final elongation at 72°Cx10min. Illustra ExoProStar was used for enzymatic purifications. Minisequences were obtained using ABI-Prism SNaPshot® Multiplex kit 1X, 0.1-1uM each probe and 1.5ul of purified PCR product. SBE probes were run on an ABI3130 genetic analyzer and sized by Genemapper V.3.2 Software. SNaPshot multiplex reaction enabled us to accurately identify the six selected SNPs, further validated by Sanger sequencing. This means that in Ecuador the cost per SNP genotype by traditional Sanger sequencing was reduced by SNaPshot Multiplex technique in 1:12. This can be reduced even more if other markers are included in the same reaction. With this system Latin American developing countries can access to non-expensive methods for DNA typification and potentially applicable to multiple fields such as population's genetics, molecular diagnostics and especially for forensic genetics' applications.

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251. DEVELOPMENT OF AN INNOVATIVE APPROACH TO HUMAN DNA QUANTIFICATION ANALYSIS

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Recently introduced Quantifiler™ Trio human DNA quantification kits are more sensitive, resistant to inhibitors, include a more robust DNA standard, and provide sample quality and quantity information. The result of these changes is that the laboratory can more accurately predict downstream STR results including deciding whether to continue with STR analysis, which STR kit to use, and how much DNA to add to the STR reaction. Although improved and optimized, accurate quantification results still depend heavily on the standard curve generation and metrics. To further increase efficiency and decrease variability a flexible Virtual Standard Curve (VSC) feature was developed within the HID Real-Time PCR Analysis Software v1.3. VSC functionality allows the user to input standard curve quality metrics for each quantification target and analyze or re-analyze data with the user-defined values. Ultimately, this feature reduces quantification variation by negating the variation introduced during the creation and addition of the standard curve dilution series. This approach also increases productivity by allowing for the addition of more samples to each plate and saves analyst time. Three sources of variation were assessed, including multiple users, lots of kits, and QuantStudio™ 5 Systems. The use of optimized VSC protocols demonstrated how to decrease quantification variability while increasing efficiency. In addition, the impact of quantification values determined using a VSC versus in-plate standards on downstream GlobalFiler™ kit STR results was evaluated. This study demonstrates a VSC can be utilized in a forensic DNA laboratory to improve accuracy and increase efficiency while not impacting STR results.

252. DEVELOPMENT OF THE NGM DETECT™ KIT FOR GENOTYPING THE EUROPEAN STANDARD SET OF MARKERS WITH A NOVEL MULTIPLEX DESIGN AND ENHANCED PERFORMANCE

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STR detection assays for forensic DNA typing are constantly evolving, with novel design improvements functioning to enhance and advance existing systems in a variety of ways. In the case of the new NGM Detect™ Kit, such improvements have greatly enhanced the ability of European forensic laboratories to obtain genotype information from markers of greatest interest, particularly in the face of compromised or low-concentration DNA samples. This new assay prioritizes SGM Plus™ Kit loci by reducing amplicon sizes in a marker set and layout complementary to that of the NGM SElect™ kit -- achieved through extensive primer redesign and implementation of a sixth dye channel. This casework kit: is highly concordant (>99.5%) to the NGM SElect™ kit; includes a Y indel marker; and possesses a new internal quality control (IQC) system that signals when a reaction has been compromised. The kit has also been designed for greatly reduce reaction times (<60 mins.), as well as increased sensitivity, by way of newly developed chemistries and increased sample input volume (15µL). NGM Detect™ has demonstrated the ability to amplify a diverse range of sample types, representative of the types of evidence submitted to operational forensic laboratories; and in comparison to the NGM SElect™ Kit, when running experimentally degraded DNA or inhibited samples, the assay was shown to produce DNA profiles with higher allele recovery, in particular from the SGM Plus™ loci by virtue of the redesigned marker layout. For Research, Forensic, or Paternity Use Only. For licensing and limited use restrictions visit thermofisher.com/HIDlicensing.

253. DIRECT AMPLIFICATION OF BIOLOGICAL EVIDENCE AND DVI SAMPLES USING THE QIAGEN INVESTIGATOR 24PLEX GO! KIT

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Omitting DNA extraction and quantitation saves valuable time and avoids sample loss in criminal investigations and disaster victim identification efforts. Based on previously described protocols [1, 2], we applied a combination of direct amplification treatments to various sample types in combination with the Qiagen Investigator 24plex GO! Kit. This “megaplex” kit was designed for direct PCR of DNA references, and validated for use with a Qiagen lysis buffer. Preliminary experiments resulted in different approaches for each sample type. Missing person reference and/or post-mortem samples like hair roots, toothbrush bristles, human tissue (fresh and paraffin embedded) and bones were subjected to a 2h, 56^{O}C, Proteinase K lysis step with 2µL of the volume as PCR input. Personal effects like razors and eye glasses, and mock touch DNA evidence items were swabbed with small pieces of polyester swabs then added to the PCR master mix. Blood and saliva stains were collected using Sello tape. Adjusting the collection/pre-treatment method prior to amplification avoided negative or overblown samples and resulted in sufficient first round success to justify using the Qiagen GO kit and direct PCR in rush cases. This approach was unsuccessful for paraffin embedded tissue.

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254. DISTRIBUTION OF SPECTRAL PULL-UP WITH GLOBALFILER

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Spectral pull-up is usually caused by insufficient or sometimes excessive subtraction of spectral overlap between different color channels. This study is aimed to examine pull-up peak height ratios and the observed x-axis positions (bp) of pull-up peaks in electropherograms. Ninety five Japanese samples were amplified at 1 ng template DNA with GlobalFiler, and the electrophoresis was performed with the 3500xL and 3130*xl* genetic analyzers. The run data were analyzed with GeneMapper *ID-X* 1.4 software and threshold value was set at 40 RFU for 3500xL and 20 RFU for 3130*xl*. Pull-up peaks were searched using our original Microsoft Excel macro. With the 3500xL, 598 pull-up peaks were observed and their pull-up peak height ratios averaged 1.04±0.33% and ranged 0.260-2.80%. The pull-up from yellow to green was most frequently (220 peaks), followed by red to blue (107 peaks), red to yellow (86 peaks), and so on. Of the 598 pull-up peaks, 580 peaks were within ±1 bp apart from the parent allele

peaks. The pull-up peaks toward adjacent shorter wavelength channel (e.g. from yellow to green) tended to be observed in the left side (shorter bp) of the corresponding parent allele peaks, and vice versa for those toward adjacent longer wavelength channel. With the 3130*xl*, the pull-up peaks showed the same tendency, although they were observed more frequently (2439 peaks). We also examined the x-axis distribution of pull-up peaks by injecting J6 matrix standard with LIZ 500 or 600 v2 size standard into three 3500xL and one 3130*xl*.

255. DNA AND PROTEIN TESTING OF FIRED AND UNFIRED CARTRIDGES

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DNA on ejected cartridge casings is potentially probative, but often compromised and of low quantity. Proteins have been shown to be very stable and the additional detection of genetically variable proteins promises increased power of discrimination [1]. We applied our method of simultaneously extracting PCR ready genomic DNA and trypsin digested peptides suitable for mass spectrometry to touched fired and unfired cartridge casings. Following Wan et al. 2015 [2], touch DNA was either collected by swabbing or tape lifts, the latter avoiding moisture and oxidative reactions. Nickel, aluminum, steel and brass cartridges were tested before and after being fired. PCR testing was performed with the Identifiler Plus kit (Thermo Fisher). Peptides were separated by reversed-phase liquid chromatography using Easy-nanoLC 1000 HPLC fitted with a Q Exactive Orbitrap mass spectrometer (Thermo Scientific). Tape lifts yielded more DNA than swabbing, but most samples still had insufficient DNA for STR typing. Mass spec analysis was successful for samples negative for DNA and detected between 838 and 1903 unique peptides.

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256. DNA AUTOMATION – THE GOOD, THE BAD AND THE UGLY

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Due to the success of DNA evidence in assisting criminal investigators, forensic laboratories have faced an increasing number of samples submitted for DNA analysis. The automation of DNA analytical methods has enabled forensic laboratories to respond to the increasing demand and carry out high throughput processing of crime samples, eliminating or reducing DNA processing backlogs. The NSW Forensic & Analytical Science Service (FASS) operates a fully automated DNA laboratory currently

processing 40,000 DNA samples annually, with DNA profiles generated within a few days of sample receipt. Fast turnaround times ensure the provision of DNA results to investigators at the early stage of the investigation. However, automation of DNA analytical processes may not be straightforward, and FASS has faced challenging technical issues affecting both processing and results. Automation issues can lead to implementation delays and increase the cost of validation. This presentation follows the journey through the trials and tribulations of automation in relation to reagent specific issues encountered with processing kits. In addition, critical modifications required to minimise contamination risks associated with the design of the automated process will be demonstrated.

257. DNA PROFILING OF SPERM CELLS BY USING MICROMANIPULATION AND WHOLE GENOME AMPLIFICATION

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The use of the differential extraction method was designed to prevent mixed profiles by separated lysis of the epithelial cells and spermatozoa from intimate swabs. However, DNA from the victim can still be present in the sperm fraction and compete with the sperm's DNA. Furthermore, the suspect's DNA cannot be identified when only a minute amount of spermatozoa is present as trace. Also, differential extraction is not effective in cases when swabs contain sperm from more than one suspect. Mixed profiles could ideally be overcome by DNA profiling on single spermatozoa. However, common multiplex STR kits are not yet sensitive enough to generate DNA profiles on single cells.
The purpose of this study was to develop a method which enables DNA profiling of up to a single sperm cell. Spermatozoa were detected by using fluorescence microscopy with the SPERM HY-LITER™ PI (Independent Forensics) and isolated through micromanipulation. Spermatozoa were lysed and their DNA was pre-amplified by whole genome amplification (WGA) to generate sufficient template for PCR. To these ends, multiple WGA methods were first tested on different amounts of genomic DNA (gDNA) in combination with the PowerPlex ESX 17 ® STR system (Promega) and assessed concerning rates of allele recovery, allele drop out (ADO) and allele drop in (ADI). The WGA method with the least ADOs and ADIs was selected for use on cell material and tested for autosomal and Y-chromosomal STRs. Results of WGA performance on gDNA as well as multiple and single cells will be presented.

258. EVALUATION AND COMPARATIVE ANALYSIS ON REDUCTION OF GLOBALFILER™ REACTION VOLUME IN LOW TEMPLATE SAMPLES

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Samples with very low quantities of DNA are frequently a challenge in daily forensic genetics routine. Despite the robustness of the amplification kits currently used, it is often not possible to obtain complete genetic profiles. In these limit situations, when the maximum input volume of sample was reached in the PCR reaction, the increase of the injection time in capillary electrophoresis is frequently an alternative in an attempt to obtain a more complete genetic profile.
Seeking improvements in the methodologies used to optimize the results, and based in several published studies, we evaluated the volume reduction on amplification reaction with Globalfiler™ in low template DNA samples.
To carry out this study, several samples extracted with PrepFiler® were diluted successively to very low concentration values. All samples and respective dilutions were amplified with Globalfiler™, for both reaction volumes of 12,5uL and 25uL. Capillary electrophoresis was performed on the ABI PRISM® 3500 Genetic Analyzer and the HID files generated were analyzed using GeneMapper® ID-X v.1.4.
The results indicate that the reduction of volume in amplification reaction with Globalfiler™ can often produce higher quality profiles, resulting as an alternative to enhance allelic recovery.

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259. EVALUATION OF A 55-SNP MULTIPLEX SNAPSHOT SYSTEM FOR DETECTING FORMALIN-FIXED TISSUES

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In order to evaluate the application value of the 55-SNP multiplex SNaPshot assay system established by our lab for detecting formalin-fixed tissues, 24 formalin-fixed tissues stored at -20℃ for five years were analyzed. The concentration and degradation index of DNA was quantified with Quantifiler® Trio DNA Kit. A 55-SNP multiplex SNaPshot assay and PowerPlex® 21 system were used to amplify SNP and STR loci, respectively. Separation of PCR products was performed by capillary electrophoresis, using an Applied Biosystems 3130 Genetic Analyzer. The results showed that the degradation index of 24 specimens were ranged from 1~8. The SNP genotypes of the 24 specimens were completely consistent with the non-degraded DNA from the same patients and the successful genotyping rate was 100%. However, 33 allele dropouts were observed with STR genotyping, of which the degradation index was more than 2.6, and the fragment size of the 75.8% allele was more than 300bp. There was a negative correlation between the fragment size of STR and the allele detection rate ($P<0.05$), and a negative correlation also observed between the degradation index of samples and the allele detection

rate except for two samples with mild degradation (P<0.05). This study validated that the long-term formalin-fixed tissues were susceptible to degradation, and the 55-SNP multiplex SNaPshot assay system was more suitable for detecting these tissues than STR typing system.

260. EVALUATION OF THE VERIFILER™ EXPRESS PCR AMPLIFICATION KIT WITH REFERENCE BLOOD AND BUCCAL SAMPLES

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Short tandem repeat (STR) analysis of buccal and blood samples are often used by forensic database and paternity laboratories to generate DNA profiles. Buccal samples are often used for forensic DNA databases and paternity analysis. This noninvasive collection procedure provides a good source of DNA for samples that incorporate into the direct amplification workflow with minimal effort. With a growing number of single-source reference samples collected for processing, direct amplification is a fast and effective method to increase efficiency in a single-source reference sample workflow. By eliminating the extraction and quantitation steps, the direct amplification protocol saves time and resources.
The new VeriFiler Express kit is a 6-dye, direct amplification STR chemistry designed for use with single-source, blood and buccal reference samples collected on treated and untreated paper, cotton swabs, Copan FLOQSwabs™, and Bode DNA Buccal Collectors™. The VeriFiler Express kit amplifies all Globalfiler™ Express markers except SE33 and DYS391 and includes two sex-discrimination markers, Amelogenin and Y-indel. For customers who demand a streamlined workflow that supports increased throughput and reduced processing time, the new VeriFiler Express PCR Amplification Kit—with 25 STR markers including all CODIS core loci and two highly discriminating Penta markers—provides superior genotyping results.
In this presentation, we evaluate first pass success rate and chemistry performance when the VeriFiler Express PCR amplification kit is used with various sample collection devices. Summarized data from internal and external studies will be presented.
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261. FIFTEEN SNP-STR LOCI BASED ON CODIS AND ESS

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A SNP-STR polymorphic genetic marker consists of a STR and a SNP located in the flanking region of the STR core sequence. The SNP-STR loci were screened from autosomal STRs of U.S. Core Loci and European Standard Set, now available in Expanded U.S. Core Loci, and SE33 locus. The minor allele frequency (MAF) of SNP in a SNP-STR is required to be greater than 0.01. Nineteen SNP-STRs were obtained, in which rs79317590-D18S51, rs115388493-D19S433 and rs11908851-D21S11 were excluded because the SNP of each SNP-STR has only one genotype in East Asian. The remaining loci, containing rs17651965-CSF1PO, rs2070018-FGA, rs13413321-TPOX, rs11063971-VWA, rs4847015-

D1S1656, rs6736691-D2S1338, rs58390469-D2S441, rs17077990-D3S1358, rs25768-D5S818, rs7786079-D7S820, rs57346531-D8S1179, rs2246512-D10S1248, rs7962284-D12S391, rs9531308-D13S317, rs11642858-D16S539 and rs9362476-SE33, were designed targeted primers using the method of amplification refractory mutation system (ARMS). All pairs of primers were amplified successfully in the ARMS-PCR except for the rs11063971-VWA's. The amplicons were profiled via Genetic Analyzer ABI 3130, and verified by Sanger sequencing. The genotypes of DNA with even 0.025ng and the minor DNA component (0.05ng) in the simulated unbalanced two DNA mixture, from 1:50 to 1:500, could be detected correctly using each of the fifteen pairs of primers.

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262. GENETIC CHARACTERIZATION AND DETERMINATION OF THE NUMBER OF INDIVIDUALS BY MOLECULAR ANALYSIS IN A PREHISTORIC FINDING

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The present study focuses on the genetic analysis of skeletal human remains exhumed from a ritual tomb located in Los Cercados Chalcolithic site (3970±60 YBP) (Valladolid, Central Spain). In this burial different pottery and animal remains were found, configuring a complex ritual. We focused our attention on a human maxile and three human skulls [1].
The most striking aspect of these human remains was the different impact trauma signs on the back side of the skulls. The anthropological analysis established that the skulls were typical feminine, The bad state of preservation of the maxile did not allowed to assign this to any of the three skulls. So, it was not possible to determine the number of individuals by anthropological methodology [1].
However, we could determined the number of individuals by the genetic analysis of autosomal STRs and mitochondrial DNA on the skeletal remains. It was possible to assign the maxile to one of other three human skulls. On the other hand, we have been able to verify the sex of each individual by molecular analysis. Finally, a kinship analysis among the individuals was performed using a specific software (Familias 3.0) [2], resulting in a possible sibling relationship between two of the individuals.

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263. HIGH RESOLUTION MELTING OF SHORT TANDEM REPEATS AMPLICONS

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Currently, STR amplicons detected by capillary electrophoresis are workhorse of DNA profiling for forensic human identification and databasing. However, method requires capillary electrophoresis of fluorescently labelled amplicons after amplification, what prolongs response time and makes method laborious and expensive. Though current microfluidic approaches integrated amplification and detection steps and shorten the whole process, they did not decrease a cost of DNA profiling. We hypothesize that STRs can be genotyped after amplification using unlabelled primers and High-resolution melting (HRM), thus providing simple, fast, and inexpensive method for screening.
HRM analysis differentiates DNA amplicons based on their melting. During the post-PCR analysis, minor-groove bínding fluorophores released from dsDNA upon its denaturation by gradually increasing temperature are detected. Thus, specific self-reporting melting temperature (where number of double strand and single strand amplicons equals) and specific melting curve are established. HRM is usually used for single nucleotide polymorphisms (SNPs) genotyping but there are several reports (i.e. 1, 2) describing attempts to use HRM for STRs.
In this poster, we report our results of 96 DNAs profiled for vWA, D18S51, and D5S818 using unmodified or snapback modified primers for HRM on high temperature precision thermocycler Bio-Rad CFX96.
Acknowledgements:
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264. HIGH-EFFICIENCY AUTOMATED DNA EXTRACTION METHOD FOR DEGRADED OLD SKELETAL SAMPLES

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In case of extreme degradation, bone may be the only suitable material available for successful STR genotyping. However, relatively specialized techniques are required for the extraction of DNA from bone tissues, particularly when the bones have been exposed to adverse environmental conditions and DNA is degraded and present in low concentrations. In this study, to optimize DNA extraction procedure for old and highly degraded skeletal remains, newly developed automated DNA extraction system was compared to the traditional organic DNA extraction methods. We adopted Chemagic MSM I system for

automated DNA extraction, with optimized protocols for large volumes (range, 5-15 mL) of bone samples. DNA was extracted from randomly chosen bone samples of the victims who were killed in the Korean War and the efficiencies of extraction methods were compared by real-time PCR to measure DNA quantity and the presence of inhibitors. Success rates of genotyping were analyzed by STR genotyping, and mtDNA sequencing analysis. Real-time assays for quantification revealed that human DNA yield from skeletal samples did not show remarkable differences between two methods. However, Chemagic MSM I system showed lower concentration of co-extracted PCR inhibitors than the traditional organic extraction methods. Furthermore, STR genotyping results showed that Chemagic MSM I system is more effective DNA extraction method for old skeletal remains than organic extraction method. Therefore, here we suggest that the Chemagic MSM I is efficient automated DNA extraction system for old and highly degraded bone samples and powerful and valuable tool for identification of old skeletal remains.

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265. IDENTIFICATION OF AN EXHUMED CORPSE BY DNA EXTRACTION FROM BULB SWAB. A DISPUTED PARENTAGE CASE REPORT

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Post mortem DNA degradation makes the selection of biological samples to isolation and amplification of nucleic acids difficult and complex. Routinely it is necessary the processing of a bone sample which requires surgical removal from the deceased, refrigerated storage, and additional processing steps before DNA analysis in comparison to other ones. To overcome aforementioned issues authors describe the identification of an alleged father in a paternity test. DNA extraction method from ocular bulb decomposed material from orbital cavities in a case of a corificated 20 years old corpse enclosed in a metallic coffin, as an efficient alternative source which can be more easily collected and processed than traditional methods. In this circumstance a complete DNA profile from a ocular bulb swab was obtained using the Powerplex® Fusion kit and Powerplex® ESX17 kit. Confirmation of the DNA' profile of deceased man was carry out analysing muscular tissue from quadriceps femoris femoral and a section of a femoral bone. Hands and toenails, patellar bone and incisors were not suitable for processing. Based on the results obtained authors will be implementing material from ocular globe as a sample type for collection from decomposed remains when available.

266. IDENTIFICATION OF ORAL SWAB SAMPLES WITH THE LATEST DNA TECHNOLOGY (24 STR DNA LOCI) : A VALIDATION STUDY

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As the frequency of alleles of STR loci varies between populations, it is important to know how often genetic markers are observed in the population for interpreting the evidence obtained from the DNA correctly. The primary objective of this study is to increase the efficiency and success rate of the profiling studies for determining the paternity-kinship relationships, the identification of people in a mass disaster or biological samples found at a crime scene as well as missing and unidentified persons in a criminal case. The secondary objective of this study is to make the infrastructure studies for GlobalFiler system consisting of 24 STR loci which is increasingly used in forensic sciences and also envisaged to replace all identification systems in the near future.
In this study, swab samples taken from randomly selected 100 people (50 males-50 females) aged 18 years and older who have given informed consent to participate in the study were used. DNAs were isolated from the biological samples with the silica-based QIAamp DNA Mini Kit. DNA quantities were determined fluorometrically with Qubit Fluorometer. The DNA fragments of the desired loci of the isolated DNAs were amplified using the GlobalFiler Kit. PCR products were electrophoresed on ABI 3130 Genetic Analyzer. During the validation study, parameters such as threshold analysis study (LOD), dynamic field study, sensitivity study (LOQ), stochastic threshold study, reproducibility and repeatability, precision, mixture and contamination were determined. The allele frequencies were calculated with Arlequin software. Our genotyping results will be presented at the congress.

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267. MAGNETIC BEADS: AN ALTERNATIVE METHOD TO ENZYMATIC PURIFICATION FOR SNAPSHOT REACTIONS

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SNPs constitute a valuable tool for research in forensic genetics, population and evolution. Although

numerous kits based on the SNaPshot® technology have been developed for these fields, the RFLP technique is still the most used because of its low cost despite its low performance. Sanger sequencing is still considered the Gold Standard method for SNP genotyping. When studying SNPs outside read length (>1000bp), typing by Sanger sequencing becomes unfeasible and expensive because it requires sequencing both DNA strands in different reactions. SNaPshot® technology uses two filters of specificity: primers for multiplex PCR and probe for SNaPshot Single-Base-Extension (SBE), thus enabling the genotyping of up to 20 multiplexed SNPs located in several regions of the genome, in a single capillary electrophoresis run. Nevertheless SNaPshot® requires two purifications steps with thermo labile enzymes Exonuclease I (ExoI) and Shrimp Alkaline Phosphatase (SAP), which greatly complicates workflow and has resulted in restricted use of this efficient and informative technique in many laboratories.
This work reports the novel application of magnetic bead systems as an alternative to enzymatic purifications for SBE probes up to 100 bp. The Ampure and Cleanseq (Beckman Coulter Co.) systems allowed simpler (using basic forensic laboratory equipment as magnetic plate or a common mini-centrifuge) and faster purification of both multiplex amplicons (evidenced by a very low noise to signal ratio) and fluorolabeled SBE probes (whose recovery results in high intensity peaks). A standardized and detailed protocol is presented for validation in small to medium forensics and population genetics laboratories workflow.

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268. MEASURING HUMAN DNA DEGRADATION AND GENDER DETECTION IN FORENSIC DNA SAMPLES BY Q-PCR / HRM ANALYSIS

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Human DNA quantification, DNA degradation assessment and gender determination are key aspects in every field where human DNA analysis is required. This assay is a tetra-plex Real Time quantitative PCR reaction whose products are analyzed by HRM (high resolution melting) analysis using the intercalating dye SYTO9. The system produces four amplicons: 1- transducin (beta)-like 1, Y-linked - TBL1Y (84bp), 2- amelogenin (106/112 bp, female/male), 3- DeGraded small target DNA–DGst- (152bp) and 4-DeGraded large target DNA- DGlt- (244 bp). DNA quantitation is based on Amelogenin amplification, TBL1Y amplicon allows detecting male DNA and DGst/DGlt to assess DNA degradation level. Each fragment has different melting behavior reflected in different melting temperatures.
The q-PCR system proved good linearity in triplicates among 16 pg/ul – 50 ng/ul DNA concentration range. Adequate amplification efficiency and reaction slopes were obtained for all replicates.
After HRM analysis, four melting peaks are detected in a male DNA sample and three melting peaks if only female DNA is present. The DGst / DGlt parameter reflects the integrity of the genetic material present in the analyzed samples. There is a direct correlation between DNA damage and increased ratio of DGst/DGlt. The ability to collect these data during quantification step enables to improve the marker selection. Additionally, the use of intercalating dyes (Syto9) considerably reduces the cost of analysis compared to other detection approaches.
This q-PCR is rapid, sensitive, and a cost-effective method suitable for degraded DNA samples and applicable to any field where human DNA quantification is required.

269. METHODS FOR IMPROVEMENT OF ALLELE RECOVERY WITH THE GLOBALFILER ASSAY

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Bone samples are among the most difficult sample types encountered in forensics laboratories. The extracted DNA is often low quantity and degraded, making it difficult to obtain useful STR profiles. Recent short tandem repeat (STR) genotyping kits including the GlobalFiler™ assay are highly sensitive, robust to inhibitors and discriminating. These features of the GlobalFiler™ assay, combined with highly sensitive CE instruments including the 3500 Genetic Analyzer has resulted in useful STR profiles from previously untypeable DNA samples. However, bone samples are often still problematic, requiring modifications to laboratory methods.
To increase the amount of information obtained with the Globalfiler™ assay, laboratories may increase the PCR cycle number from 29 to 30. Use of 3500 CE instruments results in increased sensitivity and signal to noise ratios when compared with previous generation CE instruments. Laboratories often use lower instrument and dye specific calling thresholds to improve allele recovery.
To investigate the effects of PCR cycle number and reduced calling thresholds on resulting STR profiles, three test sites processed bone samples, followed by amplification with the GlobalFiler™ assay with 29 and 30 PCR cycles. The data was analyzed with both a 175 RFU threshold and a lower threshold, specific to both the instrument and the dye channel. The result is a comprehensive study comparing the effect of PCR cycle number and calling threshold on results obtained. Peak heights and allele recovery were improved. The impact on the overall DNA profile and potential analysis difficulties in distinguishing true signal from noise are also discussed
For Research, Forensic or Paternity Use Only.

270. MICRO SAMPLE SWABBING FOR REDUCED SAMPLE CONSUMPTION, INCREASED SENSITIVITY OF DETECTION AND ENHANCED INTELLIGENCE FOR PROCESSING BIOLOGICAL EVIDENCE

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Biological evidence contains very limited quantities of DNA which poses challenges for obtaining interpretable profiles. Because many traces are invisible, observation cannot be used to improve sample selection or prioritize sample analyses. An intelligent decision making process would be beneficial in enhancing typing success, reducing sample consumption, and providing cost/benefit to the laboratory, especially if it were efficient. We developed a sub-sampling technique using COPAN's microFLOQ™ Direct Swabs that is highly sensitive and consumes a very small portion of a sample. This workflow allows for direct amplification which reduces sample manipulation and risk of contamination. Although the amount of DNA obtained with the swab is small, direct amplification increases sensitivity of detection. The results show that dilute blood and saliva samples yield higher quality profiles than neat samples. Touch samples yielded DNA profiles that would not have been typeable with standard methods. The sub-sampling allows for a quick screen of the potential of obtaining a result so that a decision process is used for determination of what samples would be likely typeable with the more

routine workflow. Moreover, if the sub-sampling yields a sufficiently interpretable DNA profile, no additional typing may be necessary which can produce a faster turnaround time and overall cost reduction. Micro-sampling consumed little of a stain; for practical purposes the original sample was not compromised and routine typing could still be considered. This improved process yields results where standard methods fail to do so and thus will be a substantial benefit in providing investigative leads.

271. PCR-REVERSE BLOT HYBRIDIZATION ASSAY FOR Y HAPLOGROUP PREDICTION

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A PCR-reverse blot hybridization assay (REBA) for prediction Y chromosome haplogroup was designed to determine the world-wide major haplogroups by analyzing 9 biallelic Y chromosome markers (M9, M89, M122, M145, M175, M214, M217, P31, and $RPS4Y_{711}$). Prediction of biogeographic ancestry of a suspect became more and more important in crime investigation by analyzing DNA left at a crime scene. As foreign criminals increase, ancestry prediction can be an important clue to narrow down a suspect. The REBA can detect various Y-SNPs simultaneously using multiple probes. The REBA Y-SNP prediction kit successfully detected each SNP in 2800M male control DNA and synthesized gene for 9 SNPs, which were perfectly matched to sequencing data. Eight Korean, one Ethiopian, and several unknown mal DNA samples were successfully analyzed using REBA Y-SNP kit. A PCR-REBA is an easy and cost-saving method complementing other SNP detection methods such as SBE reaction. In conclusion, the newly developed REBA Y-SNP kit could be a useful tool for prediction of Y chromosome haplogroup.

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272. PERFORMANCE COMPARISON OF TWO MOST RECENT Y-STR MULTIPLEX SYSTEMS FOR REAL FORENSIC CASEWORK ANALYSIS

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Identifying male DNA in the mixture is still a challenge in the real casework analysis. Various methods, modifications and/or protocols have been suggested for the analysis of male female mixture and further for identifying male content in the mixture. But still it's difficult to separate and identify male content in a diverse mixture of male and female DNA. Due to varied reasons, most of the time samples contain trace amount of male DNA in comparison to female DNA. To overcome this limitation Y-STRs are being used. Recently with the development of 6 dye chemistry and new 3500 genetic analyser Yfiller plus (YFP) is launched. Another, same generation, but five dye chemistry supporting available multiplex is PowerPlex Y 23 system (PP Y23) which works on 3100 series also. Sensitivity of these two multiplex systems available for male DNA analysis in forensic case work is compared. On the basis of the study in more than 200 cases of rape, we found that the PP Y23 multiplex system works better even with the half reaction volume. In the experiments conducted, this was observed that even 8 µL PCR reaction mix of PP Y23 was able to amplify 16 pg of male DNA in mixture, however YFP was able to amplify more than 100 pg of male DNA in half reaction volume. More balanced Y-STR profiles were observed with low male DNA with PPY23. However, with YFP this was observed for a minimum 100 pg male DNA and more.

273. PREP-N-GO™: A NEW AND FAST EXTRACTION METHOD FOR FORENSIC BLOOD SAMPLES

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Forensics samples vary considerably in quality and quantity, making it particularly difficult to predict the amount of DNA available. Considering the entire procedure, one of the most important points is the extraction process (1). Such phase is absolutely decisive, since the higher the sample's handling, the greater the contamination probability.
Prep-n-Go™ (ThermoFisher™ SCIENTIFIC, Foster City, USA) is an extraction buffer that enables high quality direct PCR amplification. This buffer allows a more secure extraction because it involves a limited handling of the sample, having only 2 steps – the buffer addition and the heating of the sample. On the other hand, Prep-n-Go™ is already validated for buccal swabs samples; however, one of the most common crime scene evidence is the blood sample. So, it was our purpose to validate this technique for the blood samples extraction, by the evaluation of the quality of each obtained profile, through the observation of each allele height (in RFUs).
Both extraction and amplification were performed with different volumes, in order to understand which was the best approach to handle a forensic blood extraction with the referred buffer. For DNA amplification, we used different extract volumes, and 2 different approaches: the analysis of autosomal and Y-chromosome markers. After these initial proofs, we reproduced all the analyses in order to obtain replicated and valid results.
We consider that our results show a new and essential strategy to perform a different and safer blood extraction, given that this extraction reduces the samples manipulation.

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274. RE-ESTABLISHMENT OF THE VNTR D1S80 ANALYSIS SYSTEM AND NEW METHOD FOR ALLELIC LADDER CONSTRUCTION

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VNTR D1S80 locus genotyping has been extensively utilized because of its good resolution and suitability for PCR and detection using gel electrophoresis versus other VNTR loci although STR is currently considered the gold standard in forensic genetics. Since enactment of the statute of limitations on murder cases was abolished in the Republic of Korea in July 2015, the demand for re-analysis of DNA involved in unresolved murder cases that occurred over 15 years ago has increased. Notably, the National Forensic Service includes several recorded D1S80 genotypes detected from evidence that are regarded as crucial clues. Here, we re-established the D1S80 analysis system using capillary electrophoresis (Applied Biosystems® 3500xL Genetic Analyzer) and constructed an allelic ladder via new methodology using a single control DNA and seven primers. Although artificial owing to flanking region rather than repeat unit reduction, the method is rapid, simple, and could be applicable in any laboratory.

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275. REDUCED VOLUME FOR DIRECT PCR AMPLIFICATION OF BLOOD REFERENCE SAMPLES USING IDENTIFILER® DIRECT AND GLOBALFILER™ EXPRESS

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Polymerase chain reaction (PCR) is widely used technology in amplifying short tandem repeats (STRs). A number of these STRs are used to map out the DNA profile of a human individual. In order to co-amplify these STRs a PCR reaction including a number of primer pairs is optimized to ensure efficacy. PCR inhibitors are substances that interfere with DNA amplification and results in the generation of partial DNA or no DNA profiles in downstream processes. Over the last few decades many multiplex assays were commercially released and made available for forensic DNA profiling applications. Extraction and purification of DNA from biological samples is an important step in getting rid of inhibitors from a sample to obtain relatively purified DNA before proceeding with PCR. DNA profiling is an expensive technique and most law enforcement agencies around the world do not use it on high volume

crimes. Studies have shown that costs can be reduced by employing a different number of techniques in the processing steps that still guarantee good results. FTA® cards were developed for analyzing reference samples such as blood or saliva and helping to store the sample for long term purposes as it kills the microorganisms that comes along with biological stains which can be potential inhibitors. The aim of this research is to optimize a reduced volume PCR amplification for STR analysis of reference blood samples. This approach will help to reduce the costs of DNA profiling using direct amplification multiplex assays. A total of 130 blood samples from different individuals was collected on FTA® cards. The reaction was optimized using half volume for amplification with Identifiler® Direct and Globalfiler™ Express multiplex assays. The protocol was applied in a forensic laboratory and was able to reduce costs and account for variability of DNA amounts among reference samples.

276. REDUCED VOLUME PCR AMPLIFICATION USING THE AMPFLSTR® NGM SELECT™ PCR AMPLIFICATION KIT FOR STAIN SAMPLES

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An ongoing challenge of the forensic laboratories is increasing the sensitivity of the analyses and reducing the expenses without decreasing the quality of the results. Decreasing the total volume in the PCR reactions has advantages of increasing the sensitivity of the PCR due to better contact between PCR primers, DNA polymerase molecules, and the DNA from the samples. Another benefit is the reduction of the kit expenses. In this study, we investigated the effects of reduced PCR volumes on the resulting DNA profiles. A total of 200 pg control DNA and 200 pg DNA from crime case samples were amplified with the AmpFlSTR® NGM SElect™ PCR Amplification Kit (Thermo Fisher Scientific) in three different PCR volumes (5, 10, and 25µl). The amplificates were electrophoresed on an AB 3500xL Genetic Analyzer (Applied Biosystems®). The results were evaluated with respect to the signal intensities, amount of artefacts, number of detected alleles, heterozygosity balance, and locus balance.
We found a five times increase in the signal intensities with no significant increase in the number of artefacts using reduced volumes PCR (5 and 10 µl) both with control DNA and with DNA from crime case samples. There was no difference in the number of detected alleles. The heterozygote allelic balance, the locus balance, and the variance on these two parameters were unaffected by the reduction in PCR volume. However, the 5µl volume had a tendency of evaporating during the PCR reaction making the downstream robotic handling of the PCR product difficult.

277. SEX MOLECULAR DIAGNOSIS ON CRITICAL SAMPLES: COMPARISON OF DIFFERENT METHODOLOGIES

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One of the fundamental questions to the personal identification is the sex determination. Sometimes, the anthropological sex diagnosis is not trustworthy, specially when facing up to subadult skeletal remains, when the advanced degradation of the samples do not allow to visualize certain bone traits, or when we analyse isolated samples or body fluids stains. Because of that, it is essential to establish a reliable sex diagnosis method, and the molecular analysis constitutes a fundamental tool for this question. Nevertheless, many times, when we have to analyse critical samples, the molecular sex diagnosis could show some difficulties when dealing with low template DNA (LTD) and stochastic phenomena, like allelic dropout.
Through this study there were compared 4 different methodologies for the molecular sex diagnosis on critical samples (LTD samples). Concretely, there were analysed 70 samples from ancient human skeletal remains of different antiquity [1]. The sex diagnose was determined by 2 different autosomal STR commercial kits ((AmpFℓSTR® MiniFiler™ PCR Amplification Kit, AmpFLSTR® NGM™ PCR Amplification Kit), which includes an amelogenine gen marker [1] [2]; The other methodology contemplated the amelogenin gen amplification, but this time quantification was also performed by real time PCR (RTPCR) [3][4]. The last employed technique was based on the analysis of X chromosome markers (X-INDELs) [5]. Through these analysis, we compare the 4 different methods, evaluating advantages and disadvantages and comparing also with the anthropological sex diagnosis.

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278. SIMULTANEOUS DNA AND PROTEIN EXTRACTION USING TRYPSIN

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Protein assays can supplement DNA testing if body fluid identification is desired, or if compromised DNA results could benefit from detecting polymorphic proteins [1,2]. We are describing a method to simultaneously generate PCR ready genomic DNA and peptides suitable for mass spectroscopy analysis. The incubation buffer consists 0.01% Protease Max (Promega), and 5mM DTT in 50mM NH_4CO_3. The extraction proceeds with a 56°C denaturation step, and a 3 hour 37°C trypsin digestion.

PCR testing was performed with the Identifiler Plus kit (Thermo Fisher). 5uL of extract or flow-through were separated by reversed-phase liquid chromatography using Easy-nanoLC 1000 HPLC and Q Exactive Orbitrap mass spectrometer (Thermo Scientific). Volumes were scaled based on the size of the stain or swab cutting. For incubation volumes >50uL the extracts were concentrated using Microcon 100 filter units (Millipore). The DNA remained in the concentrate, while digested proteins passed through the membrane. For samples incubated in 50uL or less, samples were split and submitted to PCR or MS analysis without further purification. DNA yields were similar to DNA extraction with proteinase K and full STR profiles could be obtained. The number of unique peptides detected for fingerprints on glass ranged from 1227-2309.

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279. SIMULTANEOUS HUMAN IDENTIFICATION AND ANIMAL SPECIES DISCRIMINATION EMPLOYING DEVELOPED BLOCKING-PCR FROM HUMAN/ANIMAL MIXTURE SAMPLE

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Forensic casework samples for human short tandem repeat (STR) typing are often contaminated by animal DNA due to various environments and situations. In addition, DNA obtained from casework samples is limited in quantity in many cases. Herein, we developed a novel animal species identification method that uses PCR products amplified by commercial Human Identification (HID) kit from a human/animal mixture sample. Validation studies for HID kit have reported that non-specific peaks generated from some animals were observed in the molecular region lower than that of the amelogenin marker range[1,2]. First, we revealed that sequences of the non-specific peaks generated from DNA of each animal are difference among animal species. However, it is unable to analyze animal DNA using the PCR product of the HID kit when human/animal DNA is mixed. Therefore, the original polymerase chain reaction (PCR) method, blocking-PCR amplification, was developed to analyze only the sequence of the non-specific peaks from a human/animal mixture sample. Blocking-PCR was designed to amplify only animal DNA by which an oligonucleotide probe binds to specific human amelogenin to prevent amplification of non-target human amelogenin DNA. Our method that uses the remainder of the PCR product amplified by HID kit makes simultaneous examination of STR typing and animal species identification possible even if DNA is limited quantities. Actually, our method was useful for a real casework sample for HID.

1. AmpFlSTR® Identifiler® PCR Amplification Kit USER GUIDE, Life technologies, Carlsbad, CA, 2012.
2. AmpFlSTR® Identifiler®Plus PCR Amplification Kit User's Guide, Life technologies, Carlsbad, CA, 2012.

280. SNP-STR ANALYSIS FOR NONINVASIVE PATERNITY TEST OF FETUS

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The existence of cell-free fetal (cff) DNA in maternal plasma through the process of pregnancy, makes it possible to do noninvasive paternity test for a woman who got pregnant as a result of a sexual assault. However, the cff DNA in a high background of maternal DNA could not be easily detected. The SNP-STR markers, developed for analyzing the minor component in a two-man unbalanced mixture, were used for noninvasive paternity identification during pregnancy. Six SNP-STR loci, rs4847015-D1S1656, rs6736691-D2S1338, rs25768-D5S818, rs7786079-D7S820, rs2246512-D10S1248, rs11642858-D16S539, were screened based on autosomal STRs of Expanded U.S. Core Loci, and the corresponding primers were designed by amplification refractory mutation system (ARMS) method. The primers for every loci consisted of two forward primers targeting the genotype of SNP and one reverse primer, and each forward primer was amplified with the reverse one respectively. The amplicons of the six loci were all less than 210bp. The cff DNA of plasma samples from ten pregnant women at 16 to 22-week was detected using the informative makers selected from the analysis of maternal and real paternal DNA by three multiplex SNP-STR PCR. The results demonstrate the availability of SNP-STR in the noninvasive paternity test.

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281. TARGET SEQUENCING PANEL FOR KOREAN-SPECIFIC AUTOSOMAL SNPS

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Analysis of large numbers of SNPs can assist with STR markers to increase additional discrimination power, and particularly, it can supply important evidence for kinship or ethnic identification. This study designed NGS-based target sequencing panel for 118 Korean-specific autosomal SNPs (minor allele frequencies (MAFs) of ≤0.3 in 1000 Genome Database but >0.3 in Koreans). All the selected SNPs were functionally non-significant and were in the Hardy-Weinberg equilibrium with mean MAF of 0.454 in Koreans but 0.180 in the 1000 Genomes. Target was captured by the SureSelect XT Custom capture kit (Agilent) and was sequenced using the HiSeq 2000 (Illumina). Variant calling and annotation were performed by the GATK and ANNOVAR programs. The average mean read depth of target sequencing was about 2,700X and minimum and maximum were between 1,500X and 4,000X. The sequencing was confirmed by the Sanger sequencing method. The combined power of discrimination was very high with

the match probability of 2.939×10^{-49} in Korea. Observed and expected mean heterozygosity were 0.492 and 0.484, respectively. When the SNP panel was applied to the several human groups, it was able to clearly distinguish the main races of the world except the Chinese and Japanese from the Koreans. Therefore, we believe that these SNPs could be useful as Korean- or Northeast Asian-specific forensic markers, providing additional discrimination.

282. THE APPLIED BIOSYSTEMS™ NGM DETECT™ PCR AMPLIFICATION KIT– AS PROMISING AS PROMISED?

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There are various STR kits on the market to analyze forensic case work samples. While already using some of these well-established STR kits regularly, our lab is still interested to test new kits to possibly improve successful genotyping of challenging samples. The Applied Biosystems™ NGM Detect™ PCR Amplification kit offers new possibilities by using a six color approach, newly designed primers and a rearrangement of the systems compared to the AmpFlSTR® NGM SElect™ PCR Amplification Kit. Before we apply a new kit in our routine work it undergoes a thorough internal validation process. Validating NGM Detect™ resulted in the following conclusions: Whereas NGM Detect™ performs nicely with degraded or inhibited samples and shows full repeatability, we did not experience the internal quality control (IQC) as an advantage. The peak height of the IQC is often too high compared to the peak height of our samples which makes it necessary to manually adjust the scale of the 6-FAM channel for every low concentration DNA-profile. Additionally, NGM Detect™ exhibited imbalances in peak heights for heterozygous loci, even in samples with high DNA concentrations. To conclude, we will use NGM Detect™ as a supplement kit to complement missing systems from the NGM SElect™ Kit. In addition to the validation experiments a concordance study is being executed using 1498 samples consisting of buccal swabs, blood and tissue samples described in a publication by Dion et al. [1].

1. D. Dion, A. Sulzer, A. Kratzer: Concordance testing with the new STR kits and the influence on the Swiss National DNA database, FSIGSS 3 (2011), e85-e86

283. THE DEVELOPMENT AND VALIDATION OF HOMEMADE STR PCR KIT COMPOSED OF EXPANDED 20 AUTOSOMAL LOCI

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It is not too much to say that the history of DNA typing has come along with STR technologies. Recently, many nations including US are expanding STR loci used for case work and offender DNA database to enhance discrimination power regarding DNA mixtures or low template DNA with partial profile. Also in Korea, we are about to expand national STR typing system from 13 to 20 autosomal

STR loci.
We introduce homemade STR PCR kit, named as Kplex-23, encompassing all the loci included in expanded CODIS and ESS system. They are D1S1656, D2S441, D5S818, D2S1338, D3S1358, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, TH01, TPOX, vWA, amelogenin and DYS391, Y-M175 as Y chromosomal loci.
The primer of each locus was designed to minimize the amplicon size in which even the largest one does not exceed 420 bps using 4 fluorescent dyes. The resultant multiplex PCR system has a different locus layout from other commercial kits so that it is expected to complement the locus drop-out when used combined with other kit.
Multiplex PCR condition was optimized using domestic DNA polymerase and buffer system as well as newly developed ladder which reflected alleles uniquely observed in Korean population. Through internal validation studies using control DNA, we verified not only satisfactory repeatability and concordance but also reasonable sensitivity getting all the DNA profiles without any drop-out with 100pg of template DNA.
Through further validation with forensic case samples, newly developed kit is expected to replace or supplement commercial kits for forensic usage like Korea Offender DNA database.

284. THE DUPLICATION OF VWA ALLELE MAY CAUSE DISCORDANT STR PROFILES

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The fragment analysis of forensic STR multiplexes is based on separation of PCR products by size. The analysis is based on assumption that all alleles of each marker fall in their expected range and not overlapping with alleles of other markers. However, there is always a risk to encounter unknown alleles with unexpected size falling into the range of the neighboring markers. Since positions of markers varies for different multiplexes, the alleles with unexpected size may cause discordance between multiplexes for more than one loci. We describe a case of abnormally long STR allele which exceeds the size of a longest allele which previously reported by 66 bp.
A single source sample showed triallelic genotype in D18S51 with Identifiler kit. Three additional experiments were performed with different multiplex systems to confirm the observed genotype. The third additional allele was observed in all cases but each time in different locus: PowerPlex Fusion allele 32.2 appeared in D21S11, for GlobalFiler allele 14.1 revealed in D16S539, in case of COrDIS-18 kit third allele 36.1 was detected in D21S11. The third allele was always 88 bp longer than “homozygote” vWA allele 16. The monoplex reaction confirmed that abnormal allele belongs to vWA marker. Sanger sequencing revealed 88 bp duplication of allele 16. According to the current nomenclature this allele can be named as vWA allele 32 with insertion of 24 bp.
The described case demonstrates the limitations of fragment analysis technology which should be taken into account at the stage of forensic data interpretation.

285. THE EFFECT OF DIFFERENT LEVELS OF DEGRADATION AND DNA CONCENTRATIONS ON THE QUALITY OF GENETIC PROFILES

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Degradation can be identified due to the presence of a downward slope across the electropherogram, where larger amplicons have a lower probability to be amplified. The degradation index (DI) of Quantifiler® Trio kit allows to predict the quality of the expected genetic profiles. In this study, casework samples were extracted with PrepFiler® and PrepFiler® BTA. DNA quantification was performed with Quantifiler® Trio and the results were analyzed using HID Real-Time PCR Analysis Software v.1.2. According to the quantification results, the samples were divided in two groups: **a)** similar DNA concentrations and different DI and **b)** different DNA concentrations and similar DI. All samples were amplified with GlobalFiler® and the more compromised samples were also amplified with PowerPlex® Fusion 6C, according to the manufacturer's recommendations. Capillary electrophoresis was perfomed on the 3500 Genetic Analyzer and HID files generated were analyzed using GeneMapper® ID-X Software v.1.4. As expected, a higher DI resulted in a decrease in the quality of the genetic profile for similar DNA concentrations and for similar DI, the more concentrated samples showed a more complete profile. PowerPlex® Fusion 6C provided better results than GlobalFiler® in some samples, proving to be a useful kit for the amplification of samples with higher degradation and lower concentration. Thus, the quantification results demonstrated that is necessary to consider both DI and DNA concentration to define how to proceed with samples in order to maximize allelic recovery and overcome the degradation effect.

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286. THE GLOBALFILER KIT SYSTEM: ENABLING GREATER FLEXIBILITY- RESPONSES TO FEEDBACK FROM LABORATORIES

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With the U.S. CODIS Core Loci being expanded from 13 to 20 required loci, all CODIS laboratories have either transitioned or are in the process of transitioning to a new STR multiplex system.
The GlobalFiler™ PCR Amplification Kit and GlobalFiler™ Express PCR Amplification Kit were launched in 2012 and 2013 respectively and are Thermo Fisher Scientific's NDIS approved option for US laboratories upgrading to meet the new CODIS requirements. The GlobalFiler PCR Amplification Kit was specifically developed for casework type samples, and the GlobalFiler Express PCR Amplification Kit is tailored to perform optimally in direct amplification mode to facilitate faster processing for database samples.

Since the launch of the GlobalFiler kits we have worked closely with laboratories who have implemented or evaluated one or both of the kits to gather feedback with the aim of further understanding how we could adapt the system to meet their requirements where necessary. We found that some laboratories required more flexibility in kit protocols in order to achieve desired outputs or meet efficiency goals.
In this presentation we describe workflow updates we have made in response to this feedback including development of a direct amplification protocol for reference samples using the GlobalFiler kit and optimization of the GlobalFiler Express kit protocol utilizing a PCR Enhancer to achieve higher first pass success rates with untreated paper substrates such as Bode Buccal Collectors. We have further enhanced the GlobalFiler kit workflow by evaluating higher cycle numbers to improve recovery of information from low level samples and lower cycle numbers to reduce the occurrence of off-scale data and artifacts. Data from studies conducted internally supporting these protocol updates is also presented.

287. THE SEPARATION OF MALE AND FEMALE: A COMPARISON OF SEVEN PROTOCOLS

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The differential lysis is an established method for vaginal swabs containing semen stains. However, it can be a challenge to obtain a single-source male profile when vaginal swabs contain only low amounts of spermatozoa, as DNA from the perpetrator's sperm cells tends to be obscured by the presence of an excess of DNA from epithelial cells.
There are several possibilities to perform differential lysis; commercially available protocols and laboratory-specific protocols; however these methods have never been systematically compared. In our study we compared seven different protocols (Differex™, Sampletype i-sep®DL, Sampletype i-sep®SQ, GEN-IAL® First-DNA all tissue kit, The Erase Sperm Isolation Kit, QIAcube Washing Station, in-House-method) systematically, in order to determine the efficiency of sperm separation and DNA yield.
Vaginal swabs were collected from female donor and subsequently split into halves. One half was used for differential lysis and the other was extracted with a standard extraction method without lysis to determine the improvement in female:male yield. In an initial investigation, a high concentration of semen (about 1000ng) was transferred onto vaginal swabs. The changes of female:male ratio in sperm/non-sperm fractions comparing the standard extraction samples with each of the seven protocols were used to determine efficiency of separation and the loss of spermatozoa during the separation process were measured based on male DNA concentration in the samples.
Four of the seven studied methods showed significant improvement in female:male ratio and were therefore subsequentially investigated in a further study using decreasing amounts of semen to represent realistic concentrations.

288. UPDATED AUTOMATE EXPRESS™ EXTRACTION SYSTEM PROTOCOL CARD WITH VARIABLE ELUTION VOLUMES

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As a forensic scientist, you face several hurdles in extracting DNA from challenging samples. To help increase your chances of obtaining successful STR profiles, you'll often need to obtain either more or less concentrated DNA. For samples with low amounts of DNA, lower elution volumes provide higher DNA concentrations to help obtain the maximum information from STR analysis. In samples with high amounts of DNA, such as sexual assault case samples, higher elution volumes can automatically dilute samples for downstream analysis.
We're pleased to introduce an automated solution offering a high level of sensitivity and flexibility: the new Applied Biosystems™ AutoMate™ Express™ Forensic DNA Extraction System IC Protocol Card with updated protocol scripts (V1.1), enabling a total of 7 elution volumes on our Applied Biosystems™ AutoMate™ Express™ Forensic DNA Extraction System. Using the new protocol with blood and buccal samples, we've obtained excellent and consistent STR profile quality at different elution volumes.
Key features
• Maximizes the chance of obtaining an interpretable STR profile from challenging samples
• The greatest range of elution volumes on the market: with 7 elution volumes from 20, 30, 40, 50 100, 200, and 250 µL
• Multiple elution volume options allow modulation of final DNA concentration
• Less time-consuming and more consistent than manual extraction
• Works for both Applied Biosystems™ PrepFiler™ Express standard and BTA protocols
• Improved STR peak heights with lower elution volumes and higher concentration
For Research, Forensics and Paternity only. Not for use in diagnostic procedures.

289. VALIDATION AND IMPLEMENTATION OF THE INVESTIGATOR® 24PLEX QS KIT FOR FORENSIC CASEWORK

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Advanced commercial STR multiplex systems containing the expanded CODIS core loci have been developed to achieve robust results for a wide range of inhibited and degraded forensic samples. To address these requirements and challenges, the new Investigator® 24plex QS kit (QIAGEN) was evaluated in this study which coamplifies all 23 recommended markers using 6-dye technology and incorporates an innovative internal control, the Quality Sensor (QS). The Quality Sensor, containing resultant alleles "Q" and "S", which is unique and patent pending, allows differentiation between robust amplification, degradation, inhibition, lack of amplification of DNA and failed PCR amplification. The performance of the Investigator® 24plex QS kit was evaluated using various sample types, a total of 130 samples from 25 forensic caseworks commonly encountered in our forensic laboratory during law enforcement investigation (homicide, sexual assault, robberies, criminal possession of a firearm, possession of a controlled substance, and property crimes-burglaries). The data indicates that the Investigator® 24plex QS kit produces an excellent "First Pass Success Rate" (full profile obtained with one amplification and one CE injection) with conclusive and reliable profiles, even when amplifying degraded or inhibited samples. Based on the results of this study, Investigator® 24plex QS Kit is rapid, achieves optimal results with difficult casework evidence samples and increases the detection of a minor contributor in mixtures. As an additional benefit, it improves the laboratory workflow due to the additional information given by the "Quality Sensor" and assists forensic experts in the selection of the

most appropriate rework strategy.

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290. VALIDATION OF ABI 3500XL GENETIC ANALYZER AFTER DECOMMISSIONED AND RECOMMISSIONED AT NEW PREMISES

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Anglia DNA Services moved to new premises, thus the validation of 3500xL Genetic Analyzer (Applied Biosystems) instrument was required to maintain the UKAS accreditation. The scope of this validation was to obtain the concordant results from the ABI 3500xL Genetic Analyzer at both old and new premises for the same sample sets subjected to the same accredited procedures to demonstrate the accuracy, precision and reproducibility of the procedures.
Three routine casework batches were extracted and amplified with the current accredited procedures employing QIAamp DNA Mini Kit (Qiagen) and AmpFℓSTR Identifiler PCR Amplification Kit (Applied Biosystems). The capillary electrophoresis on the ABI 3500xL Genetic Analyzer (Applied Biosystems) was conducted in triplicate for each batch at both premises. Genotyping was performed using GeneMapper ID-X v.1.3 software (Applied Biosystems).
The results showed genotyping concordance which means using the ABI 3500xL Genetic Analyzer at the new premises provides no risk regarding the accuracy, precision and reproducibility of the results. The validation also showed a very good performance of the ABI 3500xL Genetic Analyzer (Applied Biosystems) at the new premises on sensitivity, specificity, repeatability and good profile quality based on the assessment of locus peak balance and peak height.

291. VALIDATION OF SAMPLETYPE I-SEP DL FOR DIFFERENTIAL EXTRACTION AND PURIFICATION WITH PREPFILER EXPRESS IN THE AUTOMATE EXPRESS DNA EXTRACTION SYSTEM

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Genetic identification of biological samples collected from sexual-assault victims is an ongoing challenge for forensic experts. The genetic DNA analysis of gynaecological swabs or the victim's underwear often result in an imbalanced mixture of female vs male profile, with this last in an unfavourable ratio. Performing differential DNA lysis may be able to separate the male sperm cells from the female epithelial cells resulting in an enriched sperm fraction and a consequently isolated male profile. The aim of this work was to test the ability of Sampletype I-sep DL extraction system (Biotype) to separate epithelial from sperm cells in samples collected in sexual-assault victims, followed by purification with Prepfiler. In this study the I-sep was tested with mock samples containing mixtures of blood+sperm and saliva+sperm in different proportions as well as with 46 samples collected in sexual-assault victims. From these 46 samples, 22 samples resulted in an isolated male profile or with few extra alleles in minority, 2 samples resulted in an isolated but partial male profile, 5 samples did not have sufficient amount of DNA to be amplified, 16 samples had a mixture profile with the suspect's profile in minority and 1 sample resulted in the victim's isolated profile. Although the I-sep did not always provide an isolated male profile, in almost half of the casework samples resulted in a profile with the male contribution in majority. Some of these samples when previously extracted with Prepfiler did not allow the identification of any of the perpetrator's STRs.

292. VALIDATION OF THE SUREID® COMPASS HUMAN DNA IDENTIFICATION KIT

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Current commercially available STR kits look predominantly at autosomal loci, potentially missing information that could be gained through typing with Y-chromosomal markers. Using 6-dye technology, the SureID® Compass kit (Health Gene Technologies, Ningbo, China) simultaneously amplifies 33 loci: 15 Autosomal STRs, 16 Y-STRs, 1 Y-indel, and the sex determining marker Amelogenin.
Validation studies were performed in order to test the robustness and reliability of the SureID® Compass kit. Results showed the SureID® Compass kit could produce 100% profiles with the recommended input, and was able to detect a 60% profile with an input template of 125 pg. The kit was shown to be fully concordant with GlobalFiler® (Thermo Fisher Scientific, MA, USA), and showed almost complete concordance with PowerPlex 21® (Promega, WI, USA).
The ability of the SureID® Compass kit to produce analysable results from various compromised samples was assessed, and the results will be presented. In particular, the kit was able to detect Y-chromosomal DNA in mixed female/male samples at a 19:1 ratio. The inclusion of Y-chromosomal markers within the one multiplex allows for the streamlined analysis of mixed samples, and the detection of minor male contributors when present in small quantities or in the presence of excess female DNA. The stochastic effects have shown to be minimal with few instances of allele drop-out and drop-in and a consistent heterozygote balance. Through this study the SureID® Compass kit has shown to be comparable to current commercially available STR kits.

293. VALIDATION STUDIES AND FORENSIC APPLICATIONS OF INVESTIGATOR 24PLEX QS KIT

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The Investigator 24plex QS Kit (Qiagen) is used for multiplex PCR in forensic, human identity and paternity testing. The PCR simultaneously amplifies 22 polymorphic STR markers: the Combined DNA Index System (CODIS) core loci, the European Standard Set (ESS) loci, SE33, DYS391 and Amelogenin.
The Investigator 24plex QS Kit is specifically designed for rapid and reliable generation of DNA profiles from blood, buccal swabs, and forensic stains. The kit utilizes QIAGEN's fast-cycling PCR technology, allowing amplification in around 60 minutes and provides highly robust results with inhibitor-resistant chemistry.
The Investigator 24plex QS Kit Primer Mix contains two innovative internal PCR controls (Quality Sensor QS1 and QS2), which are amplified simultaneously with the polymorphic STR markers, to yield helpful information about the efficiency of the PCR and the presence of PCR inhibitors.
The Investigator 24plex QS Kit User Guide describes instrument setup and sample preparation of PCR products using the Applied Biosystems 3500 Series Data Collection Software and the GeneMapper *ID-X* Software, but an internal validation is always needed.
In this study, we report the successful analysis of amplicons obtained with Investigator 24plex QS Kit on the *3500 Genetic Analyzer*. We performed developmental validation studies following the European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) guidelines, testing several critical areas of kit performance such as sensibility, sensitivity, DNA mixtures and inhibited samples.

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294. THE STUDY OF ABO GENOTYPING FOR FORENSIC APPILICATION

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The ABO blood typing have been used for personal identification and paternity testing in forensic medicine and criminal investigations for many years, because the ABO blood typing obtained from evidentiary samples is usually one of the initial pieces of information available that could lead to identification of the suspect. Therefore, many different molecular typing methods have been reported to complement routine serological ABO blood typing in forensics.
The method that using a multiplex allele-specific primer set allows for the simultaneous detection of six SNP sites in the ABO gene(nt 261, 526 and 803) and the determination of ABO genotyping from their combinations and we applied this method to the forensic science casework samples.

The internal validation study has demonstrated that this method is a reliable method with high sensitivity, accurate and reproducible results.
This method successfully determined ABO genotyping of blood, blood stain, saliva stain, buccal swab, vaginal fluid and hair. Therefore, it can be used as a rapid screen for the forensic science casework samples before multi locus short tandem repeat(STR) profiling is performed.

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Topic 05: Body Fluids Identification, Touch DNA, Forensic Biology, Legal Medicine

295. A GEFI COLLABORATIVE EXERCISE ON DNA/RNA CO-ANALYSIS AND MRNA PROFILING INTERPRETATION

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A collaborative exercise on DNA/RNA co-analysis and RNA cell typing involving GEFI (Italian working group of ISFG) laboratories was organized in collaboration with the Netherlands Forensic Institute.
Organizers provided the 15 GEFI laboratories participating in the study with: 1) PCR primers for a 19-plex mRNA profiling assay [1], together with reference purified PCR products for each cell type targeted in the multiplex; 2) detailed protocols for DNA/RNA co-extraction, mRNA profiling, and interpretation ("scoring") of results [2]; 3) a set of 8 mock forensic stains (7 single source, one a mixture of two body fluids).
Most laboratories were able to obtain correct DNA typing results (as expected, stochastic effects were seen for low template DNA extracted from a skin stain).
As for mRNA profiling, the number of GEFI laboratories who correctly identified single source stains ranged between 11 and 13 for tissues such as blood, saliva, semen and skin. Success rates decreased for vaginal mucosa (9/15), menstrual secretions (6/15) and nasal mucosa (6/15). Five laboratories correctly interpreted the provided mixed stain (saliva + menstrual secretions). The unexpected presence of skin was reported by many laboratories in different types of stains: blood (5/15); saliva (5/15); semen (4/15). This was almost always the consequence of non-specific amplification of skin marker CDSN.
For many of the participants, this collaborative exercise represented a first exposure to DNA/RNA co-analysis. Awareness of the methodological and interpretative challenges of mRNA profiling, highlighted by the study, will be beneficial to the future implementation of this technique in GEFI laboratories.
[a]GEFI Group: F. Alessandrini, C. Bini, C. Di Nunzio, M. Di Nunzio, M. Fabbri, P. Fattorini, A. Piccinini, E. Ponzano, G. Portera, C. Previderè, A. Renieri, F. Scarnicci, A. Verzeletti

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296. AN ASSESSMENT OF THE PARADNA BODY FLUID ID SYSTEM IN A CASEWORK ENVIRONMENT

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Examinations carried out in forensic laboratories currently utilise chemical screening tests and microscopic analysis to locate and identify body fluids. However, there are currently no reliable tests to positively identify vaginal material, to differentiate between venous and menstrual blood and to confirm the presence of azoospermic semen. Furthermore, for cases involving allegations of oral intercourse, the attribution of a positive amylase reaction to saliva (as opposed to vaginal secretions) can be problematic.
LGC has developed a system that uses the ParaDNA system together with one-tube mRNA detection chemistry for body fluid identification. This can be utilized at the laboratory examination stage, prior to DNA testing, to positively identify body fluids. A series of mRNA markers were selected to positively identify saliva, vaginal secretions, peripheral blood, menstrual blood, seminal fluid and sperm cells. We have carried out an assessment of this system in an active casework environment using mock-case samples. To investigate the sensitivity and specificity of the mRNA markers, the system was tested both by direct sampling of the body fluid stain (using the ParaDNA sampling device), and using aliquots of the retained aqueous supernatant following cell-harvesting.
This presentation will provide a summary of the findings obtained from the initial experiments carried out, together with the results of a further investigation into the effect of mixed body fluid samples on the detection of the individual body fluids.

297. APPLICATION REPORTS OF BODY FLUID IDENTIFICATION BY THE DNA METHYLATION

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The DNA results from the crime scenes have been used in court to prove criminal convictions with high discrimination power. However, some crime case, the origin of the DNA analysis results could be an issue of guilt or innocence. In this study, we have tried to find out the origin of body fluids using DNA methylation method from the crime scene stains[1,2]. Case 1. In the case of indecency, the policeman was collected unknown stains at the Bus stop. The DNA results of the stain, two different men's profiles were detected in the alleged mixture of saliva and semen. After the database search, one genotype was matched arrested. But it was uncertain this profile was derived from the semen. Results of the body fluid identification by the DNA methylation, the profile of the arrested suspect was derived from the saliva, and the other profile was derived from the semen. Case 2. It was a forced molestation case, when the suspect was denying the charge, the DNA profile of the victim was detected on a cotton swab which was wiped from the fingers of the suspect. As the results of the body fluid identification, the victim's profile was derived from the vaginal fluid. Case 3. There was no semen in the rape case, but the DNA profile of the suspect was detected in the victim's underpants. As the result of DNA methylation, it was confirmed that the suspect's DNA profile was originated from the saliva. The limited of detection value was 1.25ng/ul with semen, blood, vaginal fluid and saliva. We could confirm the accuracy and possibility of the body fluid identification using DNA methylation by the Single Base Extension method.
Keyword: DNA methylation, Body fluid identification, Single Base Extension.

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298. BODY FLUID PREDICTION FROM MICROBIAL PATTERNS FOR FORENSIC APPLICATION

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The association of a DNA profile with a certain body fluid can be of essential importance in the evaluation of evidence. Several alternative methods for body fluid prediction have been proposed to improve the currently used presumptive tests. Most of them measure gene expression. Here we present a novel approach based on microbial taxonomic profiles obtained by standard 16S rRNA gene sequencing. We used saliva deposited on skin as a forensically relevant study model, but the same principle can be applied for predicting other bacteria rich body fluids. For classification we used standard pattern recognition based on principal component analysis in combination with linear discriminant analysis. A cross-validation of the experimental data shows that the new method is able to successfully classify samples from saliva deposited on skin and samples from pure skin in 94\% of the cases. We found that there is a person-effect influencing the result, especially from skin, indicating that a reference sample of pure skin microbiota from the same person could improve accuracy. In addition the pattern recognition methods could be further optimized. Although there is room for improvement, this study shows the potential of microbial profiles as a new forensic tool for body fluid prediction.

299. CASEWORK OF VAGINAL FLUID IDENTIFICATION USING DNA METHYLATION PROFILING

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DNA evidence in the form of saliva, semen, skin tissue and hair recovered from victim's body can be helpful in proving that there was physical contact between offender and victim in the investigation of sexual assaults. Nevertheless, it is of particularly importance to identify the body fluid source of the DNA where the defense may argue that the source of a victim's DNA might be from a casual touch or from saliva. Therefore, the presence of vaginal secretions provides crucial evidence in proving sexual assault in some cases. However, there are no methods for definitively identifying vaginal secretions. The *DNA* methylation detection has been recently proposed as powerful techniques for a confirmatory test for vaginal fluid as well as blood, saliva and semen because of the high specificity, compatibility with existing STR typing protocols. Here, we presented casework for identification of victim's vaginal fluid from offender's bodies, underwear and condom using multiplex methylation SNaPshot reaction. It was possible to identify for vaginal fluid in offender's fingers and penile swabs, condom and underwear samples through the vaginal fluid-specific methylation signals. Although additional validation with more casework samples will be needed, the DNA methylation profiling is expected to facilitate body fluid identification.

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300. CHARACTERIZING SANITARY NAPKINS AND DIAPER TYPES AS CRUCIAL EVIDENCES FOR SEMEN IDENTIFICATION

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Sanitary napkins and diapers are crucial evidences of a sexual assault because they may contain short-term biological fluids from the victim's intimacy, relevant in identifying a suspect. However, this is a big challenge. Body fluids are mostly captured by the superabsorbent polymers (SAPs) within the substrates which can uptake water as high as 100,000%. Until now there is no established procedure to extract the semen fluid out of the pads but the water elution of the upper layers, which are SAPs free. In the present study, we investigate, for the first time, how superabsorbent sanitary pads are, depending on its surface and composition. We characterized five main sanitary napkins and diaper types encountered in sexual aggressions: (thick *super*, thick-type and thin-type napkins, panty-liner and diapertype) to test semen sensibility (spermatozoa, PSA, semenogelin) in the upperintermediate layers (U+I). Spermatozoa quantification and male-DNA yields by optic microscopy and *Quantifiler®Trio* were described, respectively. Semen was encountered up to 1:25,000 dilution (d) for all substrates in U+I layers (<0.5% sperm recapture), whereas DNA-male cells (ng/uL) reached the highest percentage in the thin napkins (8%; up-to 1:2,500 d). The second aim was simulating the lower layers of pads, where two sodium-polyacrylate hydrogels (densities: 0.002 and 0.072 g/mL) were compared and filtered with nylon *versus* polyester 10µm-membranes within *Nao®Baskets* 2mL-vials. Elutions with H_2O, 100% isopropanol, boric and citric acids, pH 4.0 and 0.9% NaCl buffers were tested. For the simulation, the best recaptures were with water and isopropanol incubations improving the U+I yields.

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301. DETECTION AND IDENTIFICATION OF FORENSICALLY RELEVANT ORGAN TISSUE TYPES AND SKIN BY MICRO-RNA EXPRESSION ANALYSIS

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The identification of organ tissues in traces recovered from scenes and objects with regard to violent crimes involving serious injuries can be of considerable relevance and crucial for the reconstruction of the course of events in forensic investigations. Molecular genetic approaches are provably superior to histological and immunological assays in characterizing organ tissues, and micro-RNAs (miRNAs), due to their cell type specific expression patterns and pronounced stability against degradation, emerged as a promising molecular species for forensic analyses, with a range of tried and tested indicative markers.
Herein we present the first miRNA based approach for the forensic identification of organ tissues. Based on an unbiased preselection of 15 organ indicating miRNA candidates exhibiting differential expression among samples of six different forensically relevant organs and skin in a comprehensive micro array analysis we employed quantitative PCR comprising an empirically derived strategy for data normalization and unbiased statistical decision making, to validate the potential of the preselected miRNA candidates to detect and correctly identify traces of brain, kidney, lung, liver, heart muscle, skeletal muscle and skin.
We show that quantifying the expression of one miRNA per target organ can be used to reliably detect and differentiate between organ tissues even in complex mixtures and minute trace amounts and with no false positive signals from adipose, bladder, colon, gallbladder, pancreas, spleen, small intestine and stomach that were tested additionally. Also, our method is fully compatible with and complementary to forensic DNA analysis.

302. DEVELOPMENTAL VALIDATION OF THE PARADNA BODY FLUID ID SYSTEM

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LGC has recently developed a rapid mRNA-based body fluid test that runs on the ParaDNA® System, a direct sampling and instrument platform utilizing HyBeacon® hybridization probes and end-point melt detection. The ParaDNA Body Fluid ID Test can detect and identify six different body fluids; vaginal fluid, seminal fluid, sperm cells, saliva, menstrual blood and peripheral blood. Crime stains or swabs are directly sampled to collect a small percentage of material from the item for delivery into pre-prepared chemistry within each test plate. Within the instrument, a single-step RT-PCR reaction, combined with the HyBeacon detection step provides results in 90 minutes with approximately 5 minutes hands-on time.
We will describe the design and developmental validation of the ParaDNA Body Fluid ID Test based on SWGDAM guidelines. This collaborative evaluation included performance assessments of the different body fluid markers, including sensitivity, aging studies, performance on mixed samples and following exposure to various commonly used presumptive test components.
Current standard presumptive tests for body fluids can be laborious, require decisions on which to perform and are prone to cross-reactivity. By necessity, much of this work is laboratory based, involving

multiple steps to isolate, amplify and detect the resulting markers. Due to its ease of use, the ParaDNA Body Fluid ID Test could greatly support forensic processes, by offering benefits either to those considering screening of all sexual assault submissions, or to users keen to confirm the fluid from which a DNA profile originated to support testimony in court.

303. DEVELOPMENT OF A MULTIPLEX MRNA PANEL FOR IDENTIFICATION OF BODY FLUIDS

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In forensic practice biological stains are routinely preanalyzed with serological tests. Within forensic genetics, messenger RNAs (mRNAs) have increasingly gained popularity regarding their potential to distinguish between human body fluids. mRNA detection has been demonstrated to be a reliable method for positive identification of the most common biological materials obtained from forensic cases. The objective of this study is to develop a multiplex panel consisting twelve mRNA markers for their specific identification of five human body fluid stains of forensic interest. We used the following mRNA biomarkers: hemoglobin beta (HBB) and b-spectrin (SPTB) for the blood, histate 3 (HTN3) and Stathern (STATH) for saliva, Prostate Specific Antigen / Kallikrein 3 (PSA/KLK3) and Semenogelin 1 (SEMG1) for semen, Matrix metalloproteinase 7 (MMP7) and Matrix metalloproteinase11 (MMP11) for menstrual blood, Humanbeta-to defensin1 (HBD1) and mucin 4 (MUC4) for vaginal secretions, gliseraldehide-3-phosphate dehydrogenase (GAPDH) and beta 2 microglobulin (B2M) for housekeeping genes. Firstly each loci was amplified alone to check the amplification efficiency and then each 12 mRNA markers were successfully combined in a single multiplex. Finally after optimization the multiplex PCR, 12 mRNA system worked well. Therefore, we describe the development of a single multiplex mRNA-based system for the discrimination of the most common forensic body fluids.

304. DEVELOPMENT OF BIOSENSORS FOR FORENSIC ANALYSIS

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Biosensors are a class of molecules that are able to transduce an invisible biological recognition event into a measurable signal. The use of innovative optical sensing technology may enable the highly-specific, non-destructive and real-time detection of biological evidence left at crime scenes. The use of multiple recognition methods ensures that sensors can be synthesized against virtually any fluid-specific biomarker. The ability of different sensing mechanisms to identify a range of biological evidence was investigated. Sensors were tested against relative analytes both in solution and *in situ* on surfaces routinely encountered within criminal investigation. The effect of these sensors on subsequent DNA profiling strategies was also explored. Both commercial and synthesized fluorogenic peptide substrates specific to prostate specific antigen and Kalikrein 8 were first utilised for the detection of human semen and sweat, respectively.
In particular, a novel synthetic route for the rapid and efficient preparation of fluorogenic substrates utilizing Rhodamine-110 or similar fluorophores was developed. A substrate for the highly specific

detection of biological evidence within a forensic casework context was readily prepared using this method [1]. The applicability of the synthesized peptide substrate within a forensic casework context was also demonstrated. All substrates were able to detect and identify analytes in situ, even at 1:1000 fluid dilutions, giving opportunity for their use in contaminated deposits or those washed in removal attempts.

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305. EFFECT OF THE ABSENCE OF SPERMATOZOA ON MICRORNA-BASED SEMEN IDENTIFICATION

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MicroRNA-based body fluid identification systems can play a prominent role in a forensic casework. Previous studies have reported several microRNAs that are highly specific to normospermia semen[1,2]. However, it is still unknown whether these microRNAs are also detected in seminal fluid of azoospermia, and the cross-reactivity of the candidate microRNAs for the other body fluids has not confirmed in azoospermia. This study aimed to compare the expression of the multiple microRNAs between normospermia and azoospermia, and reported the expression levels of semen-relevant microRNAs in seminal fluids and the cross-reactivity to the candidates for the other body fluids.
Nine candidate microRNAs—hsa-miR-10a-5p, hsa-miR-888-5p, and hsa-miR-891a-5p for semen; hsa-miR-16-5p and hsa-miR-144-3p for venous blood; hsa-miR-203a and hsa-miR-205-3p for saliva; hsa-miR-124-3p and hsa-miR-1260b for vaginal secretion—were selected. In addition, hsa-miR-103a-3p, SNORD38b, and 5S-rRNA were used as reference RNAs for the normalization of qPCR analysis.
No significant differences in the expression of the semen-relevant microRNAs were observed between normospermia and azoospermia. The microRNAs for the other body fluids were also expressed at similar levels in both normospermia and azoospermia.
Therefore, this study suggests that the absence of spermatozoa in seminal fluid should have no effects on the expression of the microRNAs for body fluid identification.

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306. EVALUATION OF GENETIC MARKERS FOR FORENSIC IDENTIFICATION OF HUMAN BODY FLUIDS

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Body fluids are commonly recovered from crime scenes and their identification is an important part of forensic casework. Current body fluid identification techniques have limited sensitivity and specificity, require large amount of template, and are prone to contamination. Application of genetic techniques lend well to forensic casework due to their sensitivity and specificity besides being less prone to contamination. This study outlines the use of mRNA markers to identify human body fluids, including blood, semen, saliva, vaginal secretion and menstrual blood. Thirty-Two (32) body fluid specific mRNA markers were evaluated and optimised. 14-markers were selected and a capillary electrophoresis (CE) based, multiplex assay was developed to identify blood, saliva, semen and vaginal secretion samples simultaneously. The markers in the developed multiplex assay included ALAS2 and PF4 (blood), STATH and HTN3 (saliva), PRM1, TGM4, MSMB, NKX3-1 (semen), ACTB and UCE (reference genes), CRYP2B7P1, SFTA2, MUC4 and L. crispatus (vaginal secretion). In accordance with the recommendations of Scientific Working Group in DNA Analysis (SWGDAM), extensive validation of the assay was completed.
This study has led to the development of a new and novel CE based mRNA marker assay for forensic body fluid identification and demonstrates its compatibility with forensic laboratory workflow.

307. EVALUATION OF THE BMS® RAPID STAIN IDENTIFICATION MULTIPLEX KIT FOR USE IN BODY-FLUID IDENTIFICATION

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Identifying the type of body fluid on the crime scene or proof of evidence can be a great help to resolve the case. Especially in sexual assault cases, identification of the offender's sperm and victim's vaginal secretions can be a crucial clue to solve the cases. The Forensic Science Division II of Supreme Prosecutors' Office has developed markers that can identify seminal fluid, saliva, vaginal fluid, and blood using mRNA, and commercialized fluid identification kit (BMS® Rapid Stain Identification Multiplex Kit) has been made. In this study we validated its sensitivity and accuracy of body fluid identification, also tested with various mock crime scene evidences such as mixed samples and aged samples.
The kit has advantages in analyzing four kinds of body fluids at once as well as in identifying person and body fluids with co-extracted DNA / RNA. Furthermore, we studied the one-step RT-PCR method which does not need separate cDNA synthesis process and direct extraction buffer that makes no extra preparation process.
In addition, further development of a kit that is capable of analyzing end point PCR products with CE equipment instead of real-time PCR would be helpful with convenient experiment.

308. EVALUATION OF THE SPERM TRACKER™ FOR SEMEN STAINS LOCALIZATION ON FABRICS

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In forensic labs worldwide and more particularly in those of the French "Institut National de Police Scientifique", one of the first step in the identification of semen traces is the orientation method which rapidly enables the localization of semen traces, subsequently sampled for DNA analysis[1]. This test can be either based on auto-fluorescence of the semen traces, upon light excitation (mini-CrimeScope) and/or based on the detection of the acid phosphatase activity present in semen. This enzyme has the ability to catalyze the hydrolysis of organic phosphates like alpha naphtyl phosphoric acid, generating a reaction product that will subsequently react with a chromogen diazonium salt and induce a color change [2, 3]. A positive reaction is recorded upon rapid development (less than 15 seconds) of a purple color, but the procedure is long (30-40 minutes), needs special handling care of carcinogenic reagents and doesn't allow a precise location for small spots of seminal liquid since the detection is performed on a transferred image of the fabric.
Inversely, when using CrimeScope, the procedure is immediate but really lacks of sensitivity and often generates false positive results, giving only presumptive semen detection which have to be validate using a complementary technique [2, 4] [5].
Alternative methods were also extensively studied such as emerging spectrometric techniques applied for the forensic analysis of body fluids. These techniques include the use of ultraviolet-visible, infrared (IR), Raman[6], X-ray fluorescence[7], nuclear magnetic resonance spectroscopy and mass spectrometry[8] for investigating blood, semen, saliva, urine, vaginal fluid or sweat. Although all these spectrometric techniques seem to have a high potential to differentiate body fluids prior to DNA extraction, IR and Raman spectroscopy have shown the most promising results for discriminating stains from body fluids [9].
The aim of the present study is to evaluate the use of a new commercial product (Sperm Tracker™) specially developed in collaboration with the French "Institut National de Police Scientifique" (INPS/LPS69) for the detection of semen traces, directly on fabrics. We are presenting here the results of a comparative study between mini-CrimeScope and the newly launch product Sperm Tracker™. Evidence were given of the specificity, sensitivity and ease of use of the new product. Genetic analysis were also performed right after localization and demonstrate the full compatibility of the method with the subsequent DNA analysis.

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309. EXPRESSION CHANGES OF MICRORNAS IN MENSTRUAL BLOOD SAMPLES OF DIFFERENT MENSTRUAL CYCLE COLLECTION DAYS

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Three miRNAs (miR-141-3p, miR-497-5p, miR-143-5p) have been screened out and confirmed to be promising markers to distinguish between menstrual blood and peripheral blood by our study in which menstrual blood samples of menstrual cycle day 2 or 3 were collected and used in confirmation[1]. However, studies reported the expression levels of miRNAs might be different in menstrual blood samples of different menstrual cycle collection days. To evaluate the dynamic changes of expression levels of these three miRNAs in menstrual blood samples from different menstrual cycle collection days, 30 menstrual blood (day 1-5 of menstrual cycle) samples were collected. Total RNA was extracted using RNeasy mini kit (Qiagen, Germany) then corresponding cDNA was prepared using a TaqMan MicroRNA Reverse Transcription Kit (Life Technologies, USA) and three TaqMan Assays were run in triplicate on an ABI Prism 7500 using the TaqMan universal PCR Master Mix II without UNG (Life Technologies, USA). The study was in process. Current results showed expression levels of menstrual blood of day 3 and 5 varied most comparing with average expression levels of menstrual blood. For miR-141-3p and miR-497-5p, expression of menstrual blood and peripheral blood were not overlapped while overlapped region was observed in miR-143-5p. Bioinformatics analysis was used to search target genes of three miRNAs attempting to explain this observations.

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310. EXPRESSION DIFFERENCE OF MIR-10B AND MIR-135B BETWEEN THE FERTILE AND INFERTILE SEMEN SAMPLES

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Non-protein coding RNAs, miRNAs (microRNAs), are a class of promising molecular biomarkers for forensic body fluid identification as the very small size and their tissue-specific expression manners .A number of studies have showed that semen can be distinguished from forensic related body fluids by the semen-specific miRNAs through microassay screening and RT-RCR. Infertility is becoming a global health problem affecting 10%-15% of couples worldwide and half of the cases are due to male factors [1]. So high incidence of infertility forced the forensic researchers to consider the impact of the semen infertility on the semen identification. Hence, several kinds of semen from man infertility were collected into our research such as asthenospermia (AS) and azoospermia (AZ).The expression level of semen samples (fertile and infertile) and other common forensic body fluids were evaluated using two miRNA markers (miR-10b and miR-135b) by RT-PCR with TaqMan probe. Results showed that the two miRNAs expressed significantly higher in normal semen (NS) than other body fluids. Nevertheless, there was a distinct down-regulation in the expression of AS and AZ samples. The goal of our study was to ascertain whether the two miRNAs could distinguish semen (fertile and infertile) efficiently and accurately. This study reminded forensic workers should pay attention to samples in pathology when identifying body fluids, which would have a far-reaching significance for forensic application.

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311. FINDING OPTIMAL SAMPLE COLLECTION STRATEGIES FOR DNA/RNA CO-ANALYSIS OF FORENSIC STAINS

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Progresses in DNA/RNA co-extraction techniques have made possible the single pipeline analysis of STRs and RNA for different applications, including mRNA profiling with cell-specific markers for the identification of body fluids. However, the effects of standard DNA collection methods on RNA stability and optimal collection procedures for the retrieval of RNA from forensic stains have been seldom investigated so far.
The aim of this study was to evaluate the impact of different moistening agents applied to collection swabs on DNA/RNA retrieval rates and integrity for downstream applications.
RNase free water, absolute ethanol and a commercial RNA stabilizing solution (RNAlater®) were used to collect forensic stains (n=120) consisting of: whole blood; diluted blood treated with luminol; saliva; semen; skin.
The average RNA yield was significantly higher for swabs treated with RNAlater®, compared to water and ethanol. Accordingly, scoring of mRNA profiling results [1] showed that the expected tissue was "observed" in 95% of the stains treated with RNAlater®, compared to 85% for ethanol, and 67.5% for water.
The effect of moistening media on DNA recovery rates varied among tissues. In skin samples, in particular, the average DNA yield of swabs treated with ethanol and RNAlater® was significantly higher compared to water, always resulting in full STR profiles, whereas partial STR profiles were seen in 87.5% of the stains collected with water.
Our results indicate that the choice of collection method is an important, often-neglected step influencing the outcome of forensic DNA/RNA analysis.

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312. MICROARRAY EXPRESSION PROFILE OF CIRCULAR RNAS IN HUMAN BODY FLUIDS

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In forensic science, the reliable identification of human body fluids could provide important information for crime investigation. Circular RNAs (circRNAs), with an abundant, stable and tissue/developmental-stage-specific expression, may be as potential markers for forensic body fluid identification. In this study, microarray expression profiles of circRNAs were explored with total RNA extraction from venous blood, semen, saliva, vaginal secretion, and menstrual blood samples. As results, semen, saliva and

venous blood could be easily distinguished from each other based on the circRNAs expression profiles, but vaginal secretions and menstrual blood displayed similar signatures. This study suggested that circRNAs extraction from forensic samples was possible and the microarray screening of circRNAs could enrich the candidates for forensic body fluid identification.

313. MRNA PROFILING IN ANCIENT BLOODSTAINS

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In this work, using old bloodstains dated 50 years and 60 years back respectively, we report the durability of three blood-specific markers ALAS2, CD93, and HBB, together with two housekeeping genes (ACTB; 18S-rRNA).
A total of 20 aged bloodstain samples stored in the Institute of Legal Medicine of Ferrara, Italy, were evaluated. Samples were divided into two sets: 10 samples (60 years group) and 10 samples (50 years group).
DNA/RNA co-extraction was performed using a half of every trace. Remaining ones were tested using HemDirect Hemoglobin test (SERATEC®) to compare results. DNA/RNA were extracted using AllPrep DNA/RNA Mini Kit (QIAGEN®) adopting a modified protocol developed in the laboratory.
For the reverse transcription reaction, random primers and RETROscript (Ambion®) were used. After cDNA quantification, samples were amplified using Multiplex PCR Mastermix (Qiagen®) according to the manufacturer's instructions.
Primer sequences and concentrations were adopted from Ven den Berge et al. [1] and previously tested in an ISFG Italian Working Group - GEFI collaborative exercise.
DNA was STR typed using the AmpF*l*STR® NGM™ amplification kit (Thermo Scientific®).
Detection of all amplified fragments was performed using an ABI PRISM 310 Genetic Analyzer (Thermo Scientific®).
Results showed all bloodstains positive for mRNA identification. The most stable was HBB, returning positive results in over 80% of the 60 years and over 90% of the 50 years back samples. All membranes were all weakly positive for blood, except for five 60 years back samples. Full STR profiles were obtained for all blood samples.

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314. MRNA PROFILING: APPLICATION TO AN OLD CASEWORK

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We report on a homicide where the victim died from numerous stab wounds, with a large amount of blood had been spattered. The victim was married and so authorities questioned her husband that became the suspect.
Police inspected the house of the suspect's mother finding a white sweater inside a washbasin located

in the laundry. Sweater corresponded to the one worn by the suspect.
No bloodstains were found during examination. When luminol was applied, three areas of luminescence developed.
In order to confirm the presence of human blood, traces were sampled and tested using HemDirect Hemoglobin test (SERATEC®).
All samples were negative. Genetic analysis, accomplished with AmpF*l*STR® NGM™ amplification kit (Thermo Scientific®), revealed the presence of the victim's profile.
Fortunately, although negative results in the confirmatory test, it has been still possible to convict the suspect only by one witness evidence collected by investigators.
In relation to mRNA profiling, samples were re-analyzed in order to recover this fundamental data.
A DNA/RNA co-extraction was performed using AllPrep DNA/RNA Mini Kit (QIAGEN®).
For the reverse transcription reaction, random primers and RETROscript (Ambion®) were used. Samples were amplified using Multiplex PCR Mastermix (Qiagen®) according to the manufacturer's instructions.
Primer sequences were adopted from Ven den Berge et al.[1].
DNA was STR typed using AmpF*l*STR® NGM™ kit (Thermo Scientific®).
Capillary electrophoresis was run on an ABI PRISM 310 Genetic Analyzer (Thermo Scientific®).
Results showed all samples positive for mRNA markers used. The genetic profile from the samples was the same previously achieved.

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315. NOVEL IDENTIFICATION OF BIOFLUIDS USING A MULTIPLEX METHYLATION SENSITIVE RESTRICTION ENZYME-PCR SYSTEM

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The identification of a specific body fluid encountered in a forensic investigation can give crucial information. This identification can be aided by methylation profiles based on selected markers specific to a range of biofluids. In this study, the open database of Infinium HumanMethylation450 BeadChip was searched for markers specific for semen, vaginal fluids, saliva, venous blood and menstrual blood. A total of 8 biofluid-specific methylated markers and 2 control markers were combined into a 10-plex methylation sensitive restriction enzyme-PCR (MSRE-PCR) system. Based upon the analysis of 100 DNA samples from these 5 biofluid types, unambiguous results were obtained to identify the body fluid from which it originated. Validation studies of the developed 10-plex MSRE-PCR included sensitivity, reproducibility and mixed body fluids. Co-amplification of the established MSRE-PCR system and the microsatellite loci in AmpFlSTR® MiniFiler™ PCR Amplification Kit was performed to generate both the

methylation profile for biofluid type and the miniSTR profile. This allowed human identification and the identification of the body fluid type to be performed in a single reaction. The results of this study displayed the applicability of this 10-plex MSRE-PCR system in forensic science.

316. ORGAN TISSUE IDENTIFICATION BY MICRO-RNA EXPRESSION ANALYSIS IN REALISTIC FORENSIC TYPE SAMPLES

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Inferring the cell types present in traces recovered from scenes and objects with regard to violent crimes involving serious injuries can be of considerable relevance and crucial for the reconstruction of the course of events in forensic investigations. We established a methodological framework for the identification of organ tissue types by quantitative microRNA expression analysis [1] employing an evidence based strategy for RT-qPCR data normalization [2].
Herein we present evidence, that the method facilitates detection and differentiation of brain, liver, kidney, heart muscle, skeletal muscle and skin even in realistic forensic type samples including challenged samples (swabs containing dried and aged organ tissue material and strongly degraded native specimens long-time exposed to outdoor conditions) as well as traces generated by experimental shootings at ballistic models and mock stabbings through layers of multiple tissue types.

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317. QUANTIFICATION OF RNA DEGRADATION OF BLOOD-SPECIFIC MARKERS TO INDICATE THE AGE OF BLOODSTAINS

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Determining the time since deposition of a biological stain can provide essential information to a police investigation: indicating either when a crime has occurred or if the biological evidence was deposited at the time of a known crime event, or before/after, in order to exclude the sample. Bloodstains are one of the most important types of evidence to forensic investigators. In order to use bloodstains to estimate the time since deposition, we have used reverse transcription quantitative PCR (RT-qPCR) to show the relative expression ratio (RER) between different types of blood-specific markers, encompassing both mRNA and microRNA. Targets included three mRNA markers (HBA, PBGD, and HBB) and two

microRNA markers (miR16 and miR451), along with three reference genes (18S rRNA, ACTB mRNA, U6 snRNA). Blood samples from 10 individuals were deposited onto cotton swabs and stored at room temperature to simulate natural aging. When samples reached a series of desired age points (0 days, 3 days, 6 days, 15 days, 30 days, 3 months, 6 months and 9 months), total RNA was extracted. Interestingly, by analysing their degradation rate, individual RNAs exhibited a unique degradation profiles during the nine months storage interval, and some were shown to be significantly more stable than others.

318. QUANTITATIVE EVALUATION OF CANDIDATE GENES AND DEVELOPMENT OF A MULTIPLEX RT-PCR ASSAY FOR THE FORENSIC IDENTIFICATION OF VAGINAL FLUID

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The identification of vaginal fluid from a mixed sample containing semen provides important probative evidence of vaginal intercourse in sexual assaults. In studies on the mRNA-based identification of body fluids, vaginal characteristic genes, such as MUC4 and HBD1, have been used to identify vaginal fluid; however, these are insufficient to discriminate vaginal fluid from other body fluids because of imcomplete specificity and detectability. The aim of this study was to develop a more specific procedure for identifying vaginal fluid. First, previously reported and newly selected candidate genes were evaluated quantitatively using real-time reverse transcription-polymerase chain reaction (RT-PCR). Then, we developed a multiplex RT-PCR assay to detect probable candidates simultaneously. Each amplicon was detected and quantified by chip electrophoresis. Furthermore, we examined the specificity and robustness of the developed multiplex RT-PCR assay using various body fluids and aged vaginal fluid stains. As a result of this real-time RT-PCR assay, we selected five candidate genes—MUC4, CYP2B7P1, KLK13, ESR1, and SERPINB13—on the basis of their specificity and sensitivity. Then, the simultaneous amplification of these genes was performed successfully, and each fragment could be separated and quantified automatically by chip electrophoresis. The specificity and detectability of the multiplex detection of vaginal characteristic genes were almost comparable to those of real-time RT-PCR and it could be applied to vaginal fluid samples stored at room temperature for 1.5 years. Although discrimination criteria should be set, the multiplex RT-PCR assay developed in this study could be an effective tool for the identification of vaginal fluid.

319. QUANTITATIVE METHOD —— A CRUCIAL CHOICE ON FORENSIC BODY FLUIDS IDENTIFICATION USING MIRNAS

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Earlier we studied the influences of different quantitative methods on body fluid identification using three markers and significant difference was observed in miR-124a-3p and miR-10b-5p[1]. Afterwards two classic quantitative methods were used to evaluate the identification power of miRNAs (miR-141-3p, miR-497-5p, miR-143-5p) which were screened out by array to distinguish between menstrual blood and peripheral blood. Results showed the choice of quantitative method might get totally opposite outcomes. Three miRNAs confirmed by the two methods showed significant differences in paired-sample T-tests. High concordance between TaqMan and SYBR Green results was seen in miR-141-3p and miR-143-5p expression. For miR-497-5p, the results of the two methods showed an unexpected negative correction. That was in SYBR Green the expression of miR-497-5p in peripheral blood was higher than that in menstrual blood and results of Taqman was completely the opposite. Both methods distinguished between menstrual blood and peripheral blood using miR-141-3p expression and the ΔCq values of the two body fluids did not overlap. Using the TaqMan method, theΔCq values of miR-497-5p and miR-143-5p didn't overlap between the two body fluids. Results of this study reminded us when using miRNA to identify body fluids, it was wise to use verified method. When one miRNA was confirmed in SYBR Green, we couldn't take TaqMan for granted and vice versa.

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320. SENSITIVITY OF SEMEN IDENTIFICATION TESTS. A STUDY ON BIOLOGICAL STAINS EXPOSED TO OUTDOOR CONDITIONS FOR UP TO 365 DAYS

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Biological stains are often exposed to environmental conditions that may break down or wash away the cellular material. Such decay will strongly influence the possibility to characterize the type of body fluid and to identify the source of the biological stain. The aim of this study was to evaluate the sensitivity of methods that are commonly used for identification of semen in biological stains. Sample sets were prepared by pipetting 40 microliters of semen onto pieces of cotton fabric. One set was kept outdoor for up to one year, exposed to the climate conditions of Northern-Norway. A control set was kept indoor at room temperature. Stains from both sets were collected at defined time points and subjected to testing using the acid phosphatase test, ABA-card, RSID Semen, and SPERM HY-LITER. DNA extracted from these stains was then quantified and STR-typed.
For the stains exposed to outdoor conditions we found that acid phosphatase testing, ABAcard, RSID Semen, and SPERM HY-LITER indicated the presence of semen for up to 7, 14, 60, and 365 days, respectively. Full STR-profiles were obtained from stains exposed up to 170 days, whereas only partial STR-profiles were obtained after 365 days of exposure. For the control (indoor) stains, all methods indicated semen, and full STR-profiles were obtained for the entire test set. We conclude that SPERM HY-LITER has the highest sensitivity of the investigated semen tests under these experimental condition. Also, we found that highly informative STR-profiles can be obtained from stains even after long-time outdoor exposure.

321. SERATEC - STUDY ON THE SENSITIVITY AND SPECIFICITY OF CURRENT PRESUMPTIVE METHODS OF SEMEN DETECTION IN FORENSIC SAMPLES

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The forensic investigation of libidinous acts includes the screening of suspected semen stains by presumptive biochemical tests, which are based on the detection of semen components like the enzyme Acid Phosphatase (AP) and the Prostate Specific Antigen (PSA). There are commercial kits of presumptive tests on the market, but few have the specific application for forensic cases, which usually deals with trace evidences and mixtures. Therefore, it is preferable that the sensitivity and specificity of forensic tests be greater than clinical tests.The major goal of this study is to evaluate the sensitivity and specificity of forensic kits for presumptive detection of semen, such as the SERI AP Test and SERATEC PSA Semiquant, which have specific forensic purposes. In order to test the sensitivity of these methods, a donor sample was diluted with a dilution coefficient ranging from 1:5 to 1:300 (n = 11), applied to different substrates and tested. The results were compared with literature data and confronted with microscopy results. Both AP test and PSA Semiquant showed high sensitivity and specificity for detection of semen in samples with trace materials. The AP test reacted positive until 1:145 dilution, respecting the limit time of 60 seconds to avoid false-positive results. The SERATEC PSA Semiquant showed positive results in all dilutions tested. This work confirms the importance of presumptive tests as eliminatory tests and shows that forensic kits as the SERATEC PSA Semiquant are not only very sensitive but also a great choice of method if dealing with trace evidence.

322. SIMPLE AND RAPID IDENTIFICATION OF ORAL STREPTOCOCCI IN SALIVA BY IMMUNOCHROMATOGRAPHIC STRIP

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In this work, we have developed a simple, rapid, and user-friendly method by incorporating direct polymerase chain reaction (Direct PCR) and immunochromatographic strip (ICS) to identify oral bacteria for proving the presence of saliva in forensic samples. Streptococcus salivarius (S. salivarius) and Streptococcus sanguinis (S. sanguinis) which are commonly found in oral cavity of human were selected as targets to evaluate the applicability of bacterial DNA as a marker for the forensic identification of saliva. To directly amplify the specific genes of the targets, we performed multiplex Direct PCR which does not require any cumbersome sample preparation steps such as cell lysis, DNA extraction, and purification. The resultant amplicons produced by the Direct PCR were colorimetrically detected on the ICS which has strong advantages for DNA analysis owing to its rapidity, simplicity, user-friendliness and easy interpretation by observing a colorimetric signal with naked eyes. Through the combination of the multiplex Direct PCR with the ICS, we could simply identify the multiplex oral bacteria without need of expensive analysis equipment. We therefore suggested that this method is useful for the identification of saliva in forensic science.

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323. SKIN CONTACT STAINS IDENTIFICATION BASED ON MRNA PROFILING

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Test and identification of body fluid stains from crime scene constitutes an important part in forensic practice. Over the past decade, research on mRNA profiling for body fluid identification has made a major breakthrough, making it a viable method to identify peripheral blood, menstrual blood, semen, saliva, vaginal secretions, skin contact stains, urine, sweat, and other common body fluid. This study focus on skin contact stains and a total of 8 skin-specific mRNA markers were obtained after two rounds of screening: LCE1C, LCE2B, CDSN, LOR, CCL27, DCD, KRT2, KRTDAP. Also, a fluorescence multiplex amplification systems were constructed using skin swab samples with the minimum amount of 0.1ng RNA. While in this study the skin markers turned out to be wanting in specificity. Therefore, if such tests are administered on the identification of skin contact stain, conclusions must not be reached without thorough consideration being given to case particulars, DNA test results, and information from other physical evidence. Based on the results of this study, the application of mRNA detection technology is helpful for the deduction of skin contact stains.

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324. SUPERABSORBENT LOWER-LAYERS OF SANITARY PADS: A CHALLENGE IN SEXUAL AGGRESSION CASES

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Sexual aggression evidences are often superabsorbent sanitary pads (napkins and diapers) which are

routinely present in the forensic laboratories for semen evaluation, only from their upper layers. Our previous results indicated the need to optimize the protocol of semen analysis by also considering its extraction out of the lower core composed of superabsorbent polymers (SAPs). SAPs generate a hydrogel which traps cellular components blocking the possibility to obtain the genetic material. Simple filtration was previously proved, however, further maximization with different treatments was never assayed. We hereby compare both chemical (H2O, 100% isopropanol, TNE and pH-4 buffer) and physical applications (pressured schredding with Shredder-SG3) to maximize gel-trapped semen elutions. Analyzed variables were sperm-counting and DNA quantification after seeding over five main superabsorbent pads (n=128). Optimized centrifugation with 10µm-pore nylon-filters inserted in NAO®Baskets allowed a better separation of semen from hydrogels. Thintype napkins obtained the highest male-DNA records (64%) and best treatment was H2O for overall-substrates (1.00±0.6%,SD sperm and 26.1±25.5% male-DNA yields); sperm recapture by TNE was similar (1.13±1.2%) but with worse DNA percentage (12.02±6.9%). The highest spermatozoa yields were for panty-liners eluted with H2O, TNE and isopropanol and for diapers with TNE (~2±0.9%). Finally, the combination of shredding and isopropanol elutions significantly improved (up-to 2.8x) cellular and DNA quantifications.

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325. THE CHANGING FACE OF BODY FLUID IDENTIFICATION: CAN MOLECULAR M(I)RNA-BASED METHODS OUTDO CONVENTIONAL APPROACHES FOR THE IDENTIFICATION OF LAUNDERED SEMINAL STAINS?

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New methods for body fluid identification (BFI) have been brought into focus in the forensics field recently. The knowledge about the type of the body fluid/tissue that contributed to a trace can provide contextual insight into crime scene reconstruction and connect a suspect to a crime scene. Especially in sexual assault cases, a reliable method for BFI is crucial because it is important to verify the presence of seminal fluid. Often, victims tend to clean their underwear/bedding after a sexual assault. If, after all, they later decide to report the crime to the police, investigators usually do not send laundered items for DNA analysis, as they believe that analysis after washing is not promising anymore. As not only the individualization of traces on laundered items could be important in court, but also the type of biological material, we compared the potential of parallel coextraction of DNA and RNA from the same specimen for simultaneous BFI and STR profiling of laundered items. BFI identification included the comparison of a broad range of conventional approaches as wells as new molecular mRNA- and miRNA-based methods. The examination comprises the assessment of different fabrics and washing temperatures, as

well as multiple washing steps. Overall, our results indicate that conventional approaches show limitations for BFI of laundered stains. Furthermore, the experiments demonstrated that co-analysis of DNA and RNA of laundered items is feasible, giving the possibility to simultaneously identify the perpetrator as well as the biological character of laundered seminal stains.

326. THE DEVELOPMENT OF APTAMERS FOR BODY FLUID IDENTIFICATION

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Aptamers are short sequences of single-stranded DNA or RNA able to bind to a target antigen with high affinity and specificity as a result of unique three-dimensional structure formation. Through the systematic evolution of ligands by exponential enrichment (SELEX) process, aptamers may be selected in vitro from a random oligonucleotide library towards almost any small molecule, virus, protein or whole-cell target. As a result, aptamers are rapidly becoming an inexpensive, versatile and synthetic alternative to antibody recognition moieties and have found use within a diverse range of diagnostic assays. The presence of body fluids at a crime scene may contribute valuable evidence to a forensic investigation. However, many current fluid detection methods suffer from issues of molecular specificity. Conversely, techniques utilized to confirm the tissue source of a fluid cannot be used to locate a latent fluid stain upon an evidential surface. In this work we present the use of SELEX technology for the selection of DNA aptamers against spermatozoa and red blood cell targets for the simultaneous detection and attribution of biological fluid traces. Selection was carried out utilizing a modified cell-SELEX procedure developed by Sefah et al. [1] with monitoring of enriched aptamer pools by next generation sequencing on the Illumina MiSeq platform. It is hoped that aptamers identified during this study may be integrated into molecular biosensing assays or used as a method of cellular capture for the isolation of cellular components from a fluid mixture.

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327. TO MOVE OR NOT TO MOVE – MASSIVELY PARALLEL SEQUENCING VS RT-PCR FOR BODY FLUID IDENTIFICATION

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The dynamic nature of RNA expression in different tissues and cell types allows for RNA profiling for the identification of forensically relevant body fluids. The very nature of forensic samples brings many challenges to RNA body fluid identification – from the way samples are collected at the crime scene through to the level of degradation and abundance of RNA. With the increasing interest in massively parallel sequencing (MPS) in forensic science, we can now produce large amounts of sequencing data which allows for the analysis of RNA that is not possible using current methods like RT-PCR. We have extensively used MPS to study RNA from forensic samples and this has provided new insights and

knowledge for the detection of RNA. We are experienced at using RT-PCR for forensic casework as we have been doing this for a number of years and know all the advantages and disadvantages of this method. With our RNA MPS knowledge, we are in the process of moving towards MPS body fluid identification. However, there are many factors that need to be considered when moving from RT-PCR to MPS in a forensic setting. We will discuss our work to date on using MPS for body fluid identification from collection of samples through to the effects of chemical enhancements commonly used at crime scenes (such as fingerprint enhancement reagents), the practical implications of moving from an RT-PCR to MPS detection system and to answer the question about making the move to MPS- is it all worth it?

328. TSMP – STR PROFILE AND BODY FLUID IDENTIFICATION IN ONE ANALYSIS

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In a number of forensic cases the identification of body fluids can be very informative, especially in sex crimes. The most common source of biological material in these form of cases 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 a methylation sensitive restriction enzyme. These assays result in a tissue-specific-methylation pattern (TSMP) and allow the differentiation between semen, epithelia and other non-semen samples. In a preliminary study we optimized an already published method in our laboratory and found new markers. The aim is of this study is the implementation of secret-specific-markers into an established STR multiplex system. The free purple dye channel* of the NGM-SElect kit were used for the TSMP markers. Therefore three of our own markers (JT.1, JT.2 and KT.1) were labeled in purple and are now in process to be integrated in the autosomal multiplex assay. Our new developed STR plus secret specific multiplex allows the simultaneous detection of the STR profile und the body fluid identification of the sample.
*supported by Applied Biosystems

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329. A BAYESIAN NETWORK TO EVALUATE DNA TRANSFER IN AN ATTACK SCENARIO

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In court the questions surrounding DNA evidence is often related to the “activity” that led to the deposition of the DNA trace. The defense may provide alternative explanations about the transfer of the

defendant's DNA to the crime scene. There are several factors influencing transfer and persistence of DNA, including shedder status, type of contact and surface of the exhibit. In addition, the interpretation can be influenced by background DNA already present at a crime scene. We have used a Bayesian network to evaluate DNA evidence at "activity level" so that the probability of observing a specific DNA result can be evaluated under two competing propositions: "The defendant is the offender" (inferring direct transfer) or "The defendant is not the offender"(inferring secondary transfer). Given the two propositions, the probability of the obtained DNA result can be elicited based on factors known to influence the result.
Based on data from a simulated attack experiment we provide a Bayesian network to evaluate the probability of observing a specific DNA trace (DNA quantity, number of contributors and mixture proportions) under the given propositions, and show how shedder status of the offender and victim influences the transfer probabilities.

330. A COMPARISON OF MINITAPING AND SWABBING FOR COLLECTING DNA FROM NON-POROUS SURFACES

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With the increased sensitivity of forensic DNA profiling chemistries, forensic scientists are now able to recover and analyze trace levels of DNA left at crime scenes. Past studies have focused on the effectiveness of different swabbing methods to recover biological evidence[1,2], yet limited data are available relating to the effectiveness of various devices at recovering trace DNA from different surfaces[3]. This study investigates the efficiency of nylon-flocked swabs, cotton swabs and minitapes at recovering trace DNA. Initially, 10ng of acellular DNA was directly applied to each of three cotton and three nylon-flocked swabs to examine extraction efficiency of the swabs directly. Quantification of the extracted DNA showed that ~85% of the initially-applied DNA was recovered from the nylon-flocked swabs compared to ~50% from the cotton swabs. Next, aliquots of 10ng DNA were applied to a range of surfaces, including gun metal, plastic piping, glass, polypropylene falcon tubes, wood and leather. DNA was recovered in triplicate using each of the three devices, and then extracted and quantified. It was found that the amount of DNA lost through both recovery and extraction processes increased significantly, with <30% of the total DNA being recovered. This study suggests that techniques currently used by forensic practitioners to collect DNA may not be the most effective methods for recovering trace levels of DNA. The impact of the choice of device, type of surface sampled, and examiner experience on DNA recovery, and the implications of these findings for DNA recovery practice, will be discussed

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331. AN EVALUATION OF THE PERFORMANCE OF DNA RECOVERY FROM FIRED FIREARMS AND CARTRIDGE CASES USING MICRODIALYSIS FILTRATION

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DNA recovery from firearm, ammunition, or cartridge case had been shown to be crucial forensic tools to identify the person(s) responsible for firing them. However, owing to minimal amounts, inhibited, and degraded by high temperature during the fire, successfully recovered DNA from these evidence always get into trouble. This study focused on the success rates of DNA recovery from different locations of department-issued handguns that handled during the process of loading and firing, as well as that from ejected cartridge cases, and unspent cartridges. In order to better simulate actual casework, random individuals handling each step with normal rhythm. Double swab technique was used for DNA collection, and DNA from these items were extracted using standard Chelex-100 extraction methods. Alternatively, the supernatant from the former step were further concentrated and rinsed using Microcon centrifugal filter devices. STR analysis was conducted using the GlobalFiler® Amplification Kit. For all DNA recovered from fired firearms and cartridge cases, average of 1.4% of expected alleles were observed using the Chelex-100 methods versus the microdialysis filtration where an average of 16.2% of alleles. DNA recovery was most successful from two parts of the handguns: the slide serrations (26.2% of trials) and the magazine releases (25.0% of trials). An additional experiment was conducted to evaluate the effects of Gun Shot Residue (GSR) and lubricant which utilized for maintenance of gun on PCR inhibition. Contrary to previous similar test, we have hinted the best DNA collection areas using microdialysis filtration and the significant inhibited effect from lubricant.

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332. ANALYSIS OF STR-BASED DNA PROFILES FROM COMPLEX SUBSTRATES

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It is well-known in forensic science that fingermark traces may possess limited DNA. The flow-on effect of this is that the DNA profiling of fingermarks often yields little or no information that can be used to assist forensic investigations. For samples such as fingermarks, every effort needs to be made to reduce processes that are wasteful of DNA so that the success rate for DNA profiling is maximised.

Standard processing in most forensic laboratories involves the sample going through a DNA extraction step, which results in the loss of DNA. One possible workflow that removes the DNA extraction step involves placing the sample directly into the PCR. This process is called 'direct PCR' and has shown to be successful in other forensic applications where traditional DNA profiling failed. Furthermore, direct PCR reduces the opportunity for contamination by eliminating the multiple tube changes and additional steps required during an extraction. Consequently, there is a reduction in the cost of labour and reagents needed to process samples and a high through-put potential for case work exhibits. The work reported here examines the effectiveness of direct PCR to generate DNA profiles from fingermarks deposited on a range of complex surfaces of forensic intelligence importance. These include cartridge cases, and IED componentry (e.g., printed circuit boards, SIM cards, including componentry that has been exposed to an explosion). The quality of a DNA profile is assayed by the relative peak height of the alleles, associated artefacts, allele 'drop-in' and 'drop-out'.

333. COMPARATIVE STUDY ON METHODS OF DNA EXTRACTION AND GENOTYPING BETWEEN SINGLE PIECE OF DANDRUFF AND EZ-TAPE

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Dandruff is generally presented in criminal cases, and it contains some nucleated cells which make it possible to extract DNA for forensic uses. EZ-tape, an extraction tool for exfoliative cells, developed by Institute of Forensic Science, Ministry of Public Security, is widely used in China. Dandruff was collected by EZ-tape together with other indefinite particles, and DNA was extracted directly from EZ-tapes. In this study, we aimed to improve the utilization of samples by isolating single pieces of dandruff from the tapes. We stuck the hats worn by two volunteers with EZ-tape and scotch tape, respectively. With the help of stereomicroscope, single pieces of dandruff were collected from scotch tapes. All DNA was extracted through chelex® 100 method. STR genotypes were obtained after amplification and capillary electrophoresis. In addition, single pieces of dandruff samples were divided into three groups, large (>500μm), medium (200-500μm) and small (<200μm), according to their diameter. Results indicated that single pieces of dandruff could provide enough DNA for STR genotyping and, rare sample (4%) could be identified mixture. Besides, both contributors are detected. However, 45.8% of samples collected through EZ-tape missed one contributor and 41.7% were mixture. There was no statistical difference among the recovery of alleles for three groups in different sizes of dandruff. Therefore, dealing with single piece of dandruff may be a potential alternative for individual identification in some special forensic casework.

334. DIRECT STR TYPING FROM BULLET CASINGS

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It is not uncommon for cartridges, bullets, and casings (CBCs) to be left at a crime scene involving shootings. During loading of guns, the handler touches the CBCs and hence they can be a valuable source of touch DNA. Previous studies on development of STR profiles from CBCs resulted in very low success rates. Direct PCR has shown increased success rates with low-level DNA but has not been applied to STR typing from casings. Here, we quantified the effect of firing on DNA quantity and also evaluated direct PCR as an alternative method to STR typing from bullet casings. Buffy coat was deposited on 9mm bullets and DNA was extracted from the casings of fired and unfired bullets (N = 30 each). Firing the bullets decreased the amount of DNA recovered by 27%. For comparison of STR typing protocols, ten volunteers touched three ammunition types (9mm, 5.56mm, and 7.62mm; N = 60 bullets). Fired casings were swabbed with EO swab moistened with PBS. Total DNA obtained from fired casings were 207±523 pg. In terms of the number of alleles typed, the direct PCR protocol did not differ statistically from the conventional extraction-STR typing protocol (95% Bayesian credible intervals of 3.0–7.8 and 3.9–10.3 alleles, respectively). This study shows that direct PCR can be used as an alternative method for STR typing from bullet casings. As the direct PCR protocol is quicker and cheaper than the conventional protocol, forensic DNA laboratories may benefit from using direct PCR for bullet casings.

335. DNA TAPE FOR STUDIES ON SEXUAL OFFENCES

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The prosecution of sexual offences other than rape, associated with the sexual breach of bodily integrity, is associated with the necessity to employ the analysis of touch traces, which are secured in such crime cases. During instances when the perpetrator does not leave any traces of sperm, blood, hair or saliva in or on the body of the victim, the victims clothes or the crime scene, it is necessary to search for residual epithelial cells on the victims clothes or in places which did not have direct contact with the external environment: the inner surface of upper parts of pants, skirts, underpants and bra cup padding. The presence of suspect material in such places may be a crucial clue, since the transfer of the genetic material on such surfaces is associated with sexual offences.
We introduce a validation of collecting touch samples from the surface of clothes using the DNA TAPE®. The tape was prepared in the form of single-use bars, which were laser cut in order to allow for simple and safe collection of evidence material from clothes. The results of comparative analyses indicate that the proposed method if far more efficient compared to cutting fragments of clothes. The validation methods indicate that in 80% of cases unequivocal genetic profiles of the users are obtained from used clothes. Even in case of a short contact between the perpetrator and the victims clothes, unequivocal genetic profiles are obtained, including the range of autosomal STR loci as well as Y-STR markers.

336. DNA TESTING OF A TRACE BLOODSTAIN ON A LAUNDERED JACKET

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Recent developments in DNA technology have allowed the detection of DNA from poor quality or trace

amounts of cells. Here, we examined the possibility of DNA detection from a DNA sample exposed to severe conditions, simulating a scenario in which an assailant attempted to destroy evidence. A red jacket with a bloodstain that had been dry cleaned (organic solvent rather than water) was submitted for DNA analysis. Because the blood was invisible to the naked eye, a specific light device (Pollilight-Flare Plus, Hitachi) was used to identify the stain, as blood absorbs at a wavelength of 415 nm. Material containing the small spots of blood was cut from the jacket, submerged in lysis buffer in a 2.0-ml tube, and treated with lectin to induce DNA agglomeration. The tube was centrifuged at 22,380 × *g* and the cell pellet was resuspended in lysis buffer. Following inactivation of the lectin, DNA was extracted using a DNA IQ™ Casework Pro Kit for Maxwell 16 (Promega). Extracted DNA was used as a template for PCR analysis. DNA was typed using the PowerPlex® Fusion System (PPF; Promega). Short-range PCR was performed to generate a DNA library spanning the entire human mitochondrial genome (D-loop) (Nextera DNA Library Prep Kit, Illumina). Library sequencing was conducted using the MiSeq System (Illumina), followed by data analysis. After comparing the reference sample, an almost complete profile was obtained for two of the five blood samples examined by PPF. Furthermore, all regions of the D-loop could be analyzed by MiSeq sequencing.

337. DNA TRANSFER BY DIFFERENT PARTS OF A HAND

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Hands are a major vector for transfer of DNA that is detected at crime scenes. The DNA profiles generated from touched objects are interpreted in respect to the person of interest's association with the criminal event and/or crime scene. Different parts of a hand will contact different items in different ways. In some circumstances it may be relevant to have an awareness of similarities and differences in: DNA yields, proportional contributions to mixture profiles, and transfer, from different parts of a hand. This preliminary study analyses the DNA quantities and contributing origins of profiles generated from 14 different areas of a hand, and a corresponding handprint, after contact with a glass plate, coated with dried saliva of a known individual, followed by contact with a DNA free glass plate to leave a handprint. This was replicated by four individuals. Within the conditions of this test, different parts of a hand appear to have proportionally more DNA, more non-self DNA, and may be more likely to pick-up and transfer non-self DNA, than others (especially fingertips relative to other parts of the hand). This knowledge may be important when prioritizing samples to be targeted and when interpreting DNA profiles from an activity level perspective. This study also identified improvement opportunities in experimental design to enhance the value of future studies of this type.

338. EFFICIENCY OF DNA RECOVERY FROM FINGERPRINTS ENHANCED WITH BLACK AND MAGNETIC POWDERS

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Given the fragility of fingerprints, even after the development with suitable d*actyloscopy* techniques,

failure in identifying its authorship may occur. In these cases, studying the DNA transferred during fingerprints deposition may represent a timely approach. We aim to determine the feasibility of obtaining a genetic profile after fingerprint enhancement with black and magnetic powders commonly used by forensic experts on glass surfaces. Samples were processed with NaCl 0.9%[1] as a collection solution, lysis solution methodology for DNA extraction followed by purification and concentration with Amicon® 100 column pretreated with 1ng Poly A RNA[2,3]. Quantitative analysis was performed with Investigator Quantiplex Kit (Qiagen). A set of fingerprints was initially profiled using CS7 PowerPlex® Kit (Promega). Our results show high standard deviation values for both groups of samples examined (225.6 for black powder, 486.9 for magnetic powder) corroborating previous studies on cell donor variation. However, fingerprints enhanced with magnetic powder showed a higher DNA mean of 190pg/µL, while 120pg/µL, was found for black powder. Fingerprints enhanced with black powder returned 66% of the total of possible alleles for the chosen STR analysis kit, while 35% of alleles were obtained for prints enhanced with magnetic powder. These preliminary results indicate that for glass surfaces, black powder is a better technique of choice whenever d*actyloscopy* features does not provide a successful identification, and genetic analysis is required for this type of evidence. Genotyping of fingerprints should be continued and carried out with standard STR markers for human identification analysis.

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339. EVALUATION OF M-VAC® SYSTEM FOR THE COLLECTION OF TRACE DNA FROM MOCK CASEWORK SAMPLES

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The M-Vac system, a newly developed sample collection device based on wet vacuum technique, was validated and compared to the traditional double swab method in this study. 100-fold diluted whole bloods were sprayed on three kinds of surface materials such as cotton towel, wood panel, and plastic panel, followed by air drying. Three examiners performed the collection process using cotton swabs and M-VAC system twice per samples. To make mock case work samples, five participants put on three types of clothes which were inner ware, socks, and gloves for 12 hours. Five examiners performed the collection process using cotton swabs and M-VAC system with the mock samples. DNA extraction and DNA quantification was carried out. As a result, the M-VAC system showed higher efficiency for collection of trace DNA compared to cotton swab method. The quantity of DNA from towels was approximately 10 times higher by M-VAC system than that of cotton swabs, 2.5 times and 1.4 times higher from the wood panel and the plastic panel, respectively. The quantity of DNA from inner ware was approximately 5 times higher by M-VAC system than that of cotton swabs, 3.2 times and 1.4 times higher from socks and gloves, respectively.

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341. HAND ACTIVITIES DURING ROBBERIES – RELEVANCE TO CONSIDERATION OF DNA TRANSFER AND DETECTION

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It is becoming increasingly relevant to address activity level issues relating to mechanisms of how DNA may have become located at the collection site. DNA transferred to and from a surface upon contact can be influenced by the manner, frequency and relative timing of the contact, plus the types of substrates and their histories. It is important to understand these factors during criminal activity. People touch many things during everyday activities, and in a relatively short period of time, which may influence how DNA of a postulated source is gained and/or lost. Here we consider touch parameters (what, how, duration, frequency) of both hands during armed and unarmed robberies. Data were generated from the video recording of >50 events. During armed robberies, recorded for an average of 36 seconds, the dominant hand made an average of 3.2 touches and the non-dominant hand 7.0. The majority of touches by the dominant hand was with personal items whilst the non-dominant hand mainly touched non-personal items. During unarmed robberies, recorded for an average of 40 seconds, the dominant hand made an average of 6.1 touches and the non-dominant hand 8.1. The majority of touches for both hands was with non-personal items. These data broaden our awareness of what an individual's hand touches during a criminal act and how that may influence the likelihood of detecting a DNA profile derived from a person of interest from items of interest. Such awareness can provide greater accuracy when the likelihoods of alternative scenarios are proposed.

342. IMPROVING TOUCH DNA RECOVERY

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Recovery of touch DNA has the potential to link offenders to scenes of crime. However, touch samples by their nature do not contain large amounts of biological material, especially compared to biological fluids such as blood, semen or saliva. Consequently, more careful recovery and storage is desirable in order generate a DNA profile. The main aim of the work presented is to determine whether detergent-based wetting agents significantly increased DNA yields from touch samples when compared to water as a wetting agent. The results show that the use of the detergent-based lysis buffer led to greater DNA recovery from the fingerprints than when distilled water was used. Therefore, the use of the lysis buffer in the touch DNA sample collection enhances as much as 50% cellular recovery during swabbing and DNA stability was greatly improved. Therefore, the inclusion of a detergent in the swabbing solution can significantly increase DNA yields from samples of fingerprints collected from different substrates and improve quality even when stored at high post-collection temperatures for up to 48 h.

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343. LAUNDRY IN A WASHING MACHINE AS A MEDIATOR OF SECONDARY AND TERTIARY DNA TRANSFER

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The possibility of secondary transfer of biological material is a major concern in forensic DNA interpretation. With continuously increasing sensitivity of commercially available STR kits, minute amounts of DNA are sufficient to obtain a comparable DNA profile. Increased sensitivity makes the probability of detecting a profile from secondary transfer DNA source more likely to occur. This mode of transfer becomes more problematic in cases in which DNA evidence is the major or sole incriminating evidence.
The aim of this work was to investigate the possibility of secondary and tertiary DNA transfer during laundry. The modes of transfer tested were mixed and separate laundry of worn and unworn garments in household and public washing machines. In addition, the possibility of a background DNA carry-over from a washing machine's drum was investigated.
In the mixed (worn and unworn garments washed together) laundry experiment, 22% of samples from new unworn socks with no traceable DNA prior to experiment produced DNA profiles post laundry following amplification using the SGM Plus™ kit. However, when using a more sensitive GlobalFiler™ amplification kit, almost 100% of the samples post-laundry produced mixed DNA profiles.
In the tertiary DNA transfer experiment performed in a public washing machine (unworn garments only), no detectable DNA profiles were observed. Samples collected from the internal drum of 25 washing and drying machines did not produce detectible STR profiles. The implications of these results are discussed in the context of forensic DNA casework analysis.

344. MATERIALS AND METHODS THAT ALLOW FINGERPRINT ANALYSIS AND DNA PROFILING FROM THE SAME LATENT EVIDENCE

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The ability to process the same forensic evidence for the analysis of fingerprints (latent ridge impressions) and DNA profiling has long been considered impossible due to processing and sensitivity issues; evidence could be sent for latent examination or to the DNA section of the laboratory, but not both.
By systematically examining the procedures in these two forensic units and by a step-by-step approach to developing solutions to the procedural and sensitivity roadblocks, we have developed methods and reagents that allow the latent examiner to

(1) obtain an enhanced image and
(2) transfer the evidence to the DNA section for successful DNA profiling. These solutions include
(a) a more efficient method for collecting all of the biological material from the evidence (No DNA Left Behind),
(b) a redesigned lift/hinge card with a transparent liner that can lift the identified ridge impressions and after imaging, facilitates the recovery of the biological material on the adhesive,
(c) a general solution (JJ Lysis Chamber) for recovering DNA from adhesive evidence (e.g., electrical tape, duct tape, postage stamps) and from the new lift card,
(d) a novel DNA purification approach, subtractive purification, that relies on a new chromatography resin in a spin-column format (Xs column), and when additional sensitivity is required,
(e) post-PCR purification/concentration for a ~15-fold increase in CE sensitivity.
When combined in the presented work flow, searchable quality DNA profiles can (generally) be obtained from even a single fingerprint; the method is equally applicable to touch DNA and latents not suitable for comparison.

345. NORMALIZATION AND DIRECT AMPLIFICATION OF CASEWORK SAMPLES

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Purification of DNA from swabs often results in loss of precious sample, particularly in those swabs with low-levels of cellular material. The Casework Direct Kit provides a method for the rapid generation of lysates from casework swabs and fabric cuttings. The DNA may be evaluated with the PowerQuant® System to quantify the abundance of human DNA, determine the male/female DNA ratio, predict PCR inhibition, and assess degradation of the DNA. Based on information gained by the PowerQuant® System, samples prepared with the Casework Direct Kit facilitate the generation of high quality laboratory results by directing workflow decisions and minimizing repeat assays and/or sampling. This information allows normalization of human DNA for STR amplification and aids in selection of the appropriate PowerPlex® Systems (autosomal versus Y-STR analysis) to use. Samples flagged as inhibited by the PowerQuant® System would benefit from DNA purification to eliminate PCR inhibitors. The analyst may choose to stop processing samples flagged as highly degraded. In about 2 hours, multiple samples submitted from a single case can be screened with both the Casework Direct Kit (in less than an hour) and PowerQuant® System (about an hour) to identify which samples would generate the most informative STR profiles. Thus, integration of the Casework Direct Kit into a laboratory workflow scheme provides a rapid, cost effective means to generate high quality STR profiles from precious, low-abundance samples with minimal hands on time. We present data from touch DNA samples and mock casework applications.

346. PERSISTENCE OF WEARER DNA ON CLOTHING

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When relevant items like garments 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 or borrowed it to someone and some other person has to be related to the crime. In order to acquire a better understanding about the persistence and replacement of wearer DNA in those special scenarios we conducted different experiments to collect empirical data:
In a first experiment worn clothing and bags of participants were changed among each other and worn for 30 - 90 minutes.
In a second experiment the wearing time by the second person was prolonged and the effect noted.
We observed a complex spectrum of replacement and persistence of wearer DNA and compared it to results from previous work about persistence of trace DNA on burglary related tools [1]. Depending on the contact and the garment or bag material we observed a more or less complete replacement of the 1st wearer's profile by the 2nd wearer. Influencing factors are discussed. With this study we wish to complement the still limited findings about persistence of trace DNA.

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347. PREVALENCE OF DNA FROM DRIVER, PASSENGER AND OTHERS WITHIN A CAR OF AN EXCLUSIVE DRIVER

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Trace levels of DNA are often retrieved from vehicles to assist investigations of criminal activity. There is however often some ambiguity establishing whether the DNA was directly or indirectly deposited. There is currently little data relating to the prevalence, transfer and persistence (PTP) of DNA, within a vehicle from the exclusive driver and passengers. From a number of cars we sampled 36 regularly contacted internal sites from the driver's side through to the passenger's side, including areas of seats, seatbelts, steering wheel, front console, doors, and also the external driver and passenger door handles. The cars had a known recent history of an exclusive driver who resides with an individual and occasionally carries a passenger. Samples were collected, processed and interpreted as per routine methodologies. DNA quantities and profiles per site were compared with the profiles of the driver, their co-resident/partner and passengers. They were also evaluated against reported histories. The DNA of the driver and passengers were predominantly observed within the area they occupied, but were also seen within each other's areas. DNA of the non-driving co-resident/partner was frequently observed on the driver's side. DNA of the latest, as well as previous passengers persisted for long periods on particular sites. DNA of unknown origin was also observed in the cars. Knowledge of PTP within vehicles will assist sample-targeting to improve the generation of profiles providing probative value as well as interpretation of profiles from an activity level perspective.

348. RECOVERY OF DNA FROM REGULARLY-WORN HOODIES: CONSIDERATION OF RECOVERY METHOD AND SAMPLING LOCATION

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Items of worn clothing are routinely examined for DNA during criminal investigations. Various recovery methods can be employed, and the locations targeted often depend on clothing type. However, little research is published to inform the selection of recovery method and sampling location, and protocols vary among casework laboratories. This study uses regularly-worn hoodies to examine the effect of method and location on the DNA recovered. Four volunteers each wore a new hoodie regularly (6 hours/day, 2 days/week, washed at weekends) during two 4-week periods. At the end of each period, DNA was recovered by cutting and mini-taping the inside left cuff, half-collar, pocket and underarm fabric, and the DNA samples (n=64) were quantified and profiled. DNA quantities varied widely, irrespective of method or location, and increased from 0-20ng to 0-42ng between the two periods, possibly due to a rise in average regional monthly temperature from 7.8°C to 12.6°C. Hoodies from three volunteers gave major profiles matching those of the regular wearer, regardless of recovery protocol, with indirectly-transferred unknown DNA contributing <12% to these profiles. However, for the fourth volunteer's hoodies, although unknown DNA contributed similarly, the remaining contributions to the profiles were mixtures of DNA from the regular wearer and their romantic partner. Whilst DNA from the regular wearer was detected as the major contributor to some of these mixtures, it was also observed as the minor or equal contributor, varying among locations sampled and between replicate experiments. These findings have implications for the interpretation of DNA from regularly-worn clothing.

349. SUCCESS IN OBTAINING INTERPRETABLE DNA PROFILE FROM CARTRIDGE CASINGS USING DIFFERENT METHODS OF EXTRACTION AND DNA AMPLIFICATION KITS - COMPARATIVE STUDY

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Cartridge casings represent common evidence in a forensic laboratory, sometimes the only one. Given the opinion that high temperatures and pressure inside of firearm chambers compromise the quality of generally small amounts of DNA deposited by handling, and metals present in cartridge cases or gunshot residues could lead to PCR inhibition, casings are considered as poor source for obtaining interpretable DNA profiles and large efforts have been made to optimize procedures for sample collection and to improve DNA recovery, extraction and amplification efficiency.
Here we present a retrospective study on 682 cartridge casings from 139 real criminal cases. The aim was to present comparison of two commonly used DNA recovery methods – soaking and swabbing, as well as efficacy of two commercially available DNA amplification kits (AmpFLSTR® Identifiler® and AmpFLSTR® Identifiler® Plus kits). Overall success in obtaining interpretable DNA profiles was 12.8% (87/682), with 14% (39/279) and 14,8% (32/216) using soaking and swabbing method respectively, combined with AmpFLSTR® Identifiler® Plus, and 8,6% (16/187) using AmpFLSTR® Identifiler® kit and swabbed DNA samples.
Our data suggest that DNA recovery method (soaking or swabbing) is not a crucial thing in obtaining interpretable DNA profiles, as it was suggested in some previous studies. On the other hand, our results showed statistically significant difference ($p<0.05$) in obtaining interpretable results according to DNA amplification kit used and pointed out improved robustness and sensitivity of new generation DNA

amplification kits e.g. AmpFLSTR® Identifiler® Plus compared to old ones (AmpFLSTR® Identifiler®).

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350. THE EFFECT OF PRESSURE ON DNA DEPOSITION BY TOUCH

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Casework exhibits are routinely examined for DNA that might have been deposited by touch. Many variables affect DNA deposition by touch, such as 'shedder status', surface type, and nature of contact[1], including pressure. Depending on substrate type, pressure can increase the transfer of touch DNA between two surfaces[2]. This study uses a novel method to examine whether pressure can also affect the amount and quality of DNA directly deposited by touch. With the fingertips of one hand, volunteers exerted pressure for one minute onto a DNA-free polycarbonate board placed on top of a balance; all five fingermarks were then swabbed and combined as one sample for DNA extraction, quantification and profiling. For each hand, the area of the combined fingertips was used to determine the weight value to which to push the balance to give low, medium or high pressures of 4, 21 or 37 kPa. Volunteers used both their right and left hands at each pressure in a randomized order on each day of three non-consecutive days. Increasing the pressure between skin and surface significantly increased the amount of DNA deposited, which resulted in the detection of more alleles, from both the donor and unknown sources. No significant differences were observed in the amounts of DNA deposited between right and left hands and among different days for each volunteer. DNA amounts significantly varied between individuals at medium and high pressures, but not at low pressure. These findings shed light on the impact of pressure on touch DNA deposition.

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351. THE EFFECTS OF RECOVERY METHOD AND SAMPLING LOCATION ON DNA FROM REGULARLY-WORN HOODIES SUBSEQUENTLY WORN BY A DIFFERENT INDIVIDUAL

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In forensic casework, items of clothing are routinely examined for so-called 'wearer DNA', but does the DNA come from the regular wearer, the most recent wearer, or a non-wearer, whose DNA has been indirectly transferred to the item of clothing[1]? This study investigates the DNA obtained from regularly-worn hoodies that were subsequently worn by a different individual. Four volunteers each wore a new hoodie regularly (6 hours/day, 2 days/week, washed at weekends) during two 4-week periods. At the end of each period, the hoodies were worn by a different individual for four hours, and DNA was recovered by cutting and mini-taping the inside right cuff, half-collar, pocket and underarm fabric. Samples (n=64) from both experiments were quantified (0.5-28ng and 0.2-154ng) and profiled, with surprisingly reproducible DNA profiles for each volunteer. Results from one volunteer's hoodies displayed a clear effect of the recovery method on the DNA profiles obtained: cut-outs gave major profiles from the regular wearer (56-97%) and minor profiles from the second wearer (3-38%), whereas mini-tapes gave major profiles from the second wearer (82-95%) and minor profiles from the regular wearer (5-15%). Additional indirectly-transferred unknown DNA was also observed at ≤15%. Results from the other hoodies showed no effects of recovery method or location. Major profiles from the second wearer were observed for all samples from one volunteer's hoodies, but the hoodies of the other two volunteers only showed major profiles from the regular wearer. The implications of these findings for the interpretation of 'wearer DNA' will be discussed.

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352. THE INFLUENCE OF FINGERPRINT POWERDS IN THE GENETIC PROFILES

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DNA analysis has been used as a tool in criminal investigations to produce forensic evidence, even in situations in which the amount of DNA is low. The fingerprint revelation technique uses fingerprint powders to reveal latent fingerprints, allowing the identification of surfaces touched by human skin. The aim of this study was to evaluate the possibility of using the fingerprint powders as screening tools for collecting biological material in touch DNA samples, obtaining good quality genetic profiles. Five different objects related to crimes: firearms, ammunition cartridge, kitchen knife, steering wheel and gear shift were proceeded to DNA analysis. We analyzed the amount of powder adhered to the impressions produced by different parts of the hands and we measured the correlation between powders and the DNA recovered. The results showed that the amount of powder recovered differed according to the region of the hand which produced the fingerprint and the amount of adhered powder was directly related to the amount of DNA obtained. In the tested objects, it was possible to collect good amounts of DNA in the steering wheel, and in reasonable quantities in the firearms, gear shift and kitchen knife. The cartridge ammunition was the only object which was not possible to recover DNA in sufficient amount to produce quality genetic profiles. Therefore, we conclude that fingerprint powders can be used as tools for choosing the right regions to collect biological material. Samples collected along with fingerprint powders are able to produce genetic profiles with quality for genetic analysis.

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353. TOUCH DNA LOCALIZATION AND DIRECT PCR: AN IMPROVED WORKFLOW FOR STR TYPING FROM IMPROVISED EXPLOSIVE DEVICES

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Improvised explosive devices (IEDs) are used for terrorism worldwide. Bomb assemblers could leave touch DNA on IED surfaces. However, STR typing from IED evidence results in mostly partial profiles and no profiles. Part of this is due to the inefficient DNA collection process. Forensic scientists cannot directly locate the areas where DNA are left and have to rely on educated guesses. In order to increase the chance of obtaining an interpretable STR profile, we developed a method to locate touch DNA using a proprietary DNA detection reagent and a portable light source. Spraying the reagent on evidence surface followed by illumination using the light source resulted in differentiable areas of high and low touch DNA concentrations. On different substrates, including mock IED evidence, swabbing the reagent along with the touch DNA and performing direct PCR yielded significantly more interpretable STR profiles when compared to conventional "blind" DNA collection and conventional DNA extraction and STR typing (p<0.05). Spiking of the reagent into PCR reactions showed no inhibitory effects. Stability experiment suggested that the reagent is stable at -20°C for up to almost two months with multiple freeze-thaw cycles. The developed method (reagent, light source, and direct PCR) has the potential to transform the way forensic scientists work with evidence potentially containing touch DNA.

354. TRANSFER AND PERSISTENCE OF DNA AS A FUNCTION OF THE SHEDDER STATUS

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Forensic DNA experts are nowadays increasingly faced with questions concerning "activity level" at court. To answer such questions a better understanding of DNA transfer mechanisms and the persistence of DNA is mandatory. Moreover, the individual ability of leaving traces, the so called "shedder status" seems to play a role.

In the present study we tried a classification of 31 individuals in “good shedders” and “bad shedders” based on the quality and quantitiy of DNA deposited during a single contact with a plastic item. To simulate indirect transfer scenarios, handshake experiments were performed by a combination of "good” and “bad shedders”. The results showed that in some cases the “bad shedder”, as the depositor, is able to transfer DNA from the “good shedder” partner immediately after handshake in such an extent that the partner could be detected as the major contributor. With some delay of 10 to 20 minutes between handshake and touching the item, the analysis almost results in mixtures.
Further investigations regarding DNA persistence on personal items interestingly showed that the DNA of a “bad shedder” owner can be displaced by a second user within short time, if the second user is a “good shedder”.

355. TRANSFER OF PICKED-UP DNA TO COTTON PLATES

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DNA is readily transferred to a knife handle by hands during a stabbing action and DNA existing on the handled knife-handle is readily picked-up during the action and transferred to a subsequently handled object. We repeated a part of an earlier study where instead of placing a handprint on five DNA-free glass plates post handling of a knife-handle, participants placed handprints on five consecutive cotton plates. Far less DNA was able to be collected from the cotton plates than from the glass plates. This appears to be due to less efficient recovery from cotton plates. DNA from the previous handler(s) of the knife was observable on some subsequently touched cotton plates. Sometimes not on the initially touched plates but on those touched later in the sequence, pointing to potential impacts of different manners of contact. The proportion of this relative to the depositor’s DNA was on average <10%. Where there were multiple previous handlers of the knife, DNA of the most recent handler(s) tended to be more prominent than earlier handlers, within the profiles derived from the cotton plates. As per prints left on glass plates, the total and transferred amounts of DNA tended to decrease as more cotton plates were touched subsequent to picking-up foreign DNA from previously touched knife handles. The substrate of the item contacted impacts on the yield and detectability of transferred DNA. More studies are required to increase our understanding of the impacts different substrates have on prevalence, transfer, persistence and recovery.

356. TYPING DNA PROFILES FROM PREVIOUSLY ENHANCED FINGERPRINTS USING DIRECT PCR

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DNA from fingermarks can be crucial evidence in forensic cases where partial or smudged prints are

obtained and hence cannot be used for classical fingerprinting. Typing nuclear STR DNA markers from previously enhanced fingermarks provides an alternative method of utilising the limited fingermark deposit that can be left behind during a criminal act. Dusting with fingerprint powders is a standard method used in classical fingermark enhancement and can affect subsequent DNA data. The ability to generate informative DNA profiles from powdered fingerprints using direct PCR swabs was investigated. Omitting the extraction step will, for many samples, be the key to success if there is limited sample DNA. DNA profiles were generated by direct PCR from 160 fingermarks after treatment with commonly used fingerprint powders. This was achieved by a combination of an optimised double-swabbing technique and swab media, omission of the extraction step to minimise loss of critical low-template DNA, and additional AmpliTaq Gold® DNA polymerase to boost the PCR. Ninety eight out of 160 samples (61 %) were considered 'up-loadable' to the Australian National Criminal Investigation DNA Database. The method described required a minimum of working steps, equipment and reagents, and was completed within 4 hours. Direct PCR allows the generation of DNA profiles from enhanced prints without the need to increase PCR cycle numbers beyond manufacturer's recommendations. Particular emphasis was placed on preventing contamination by applying strict protocols and avoiding the use of previously used fingerprint brushes

357. WHO TOUCHED THAT WOMAN? OPPORTUNITY AND LIMITS OF TOUCH DNA

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In January 2016 an American girl was found dead in his apartment with signs of strangulation. During the autopsy same samples from the victim's body was carried out using sterile swabs, including the lesions on the neck, some bruising on the wrists, on the chest, on her hair and under fingernails. From each of these swabs DNA was extracted and quantified by real-time PCR using Quantifiler Trio. In each samples a mixture of female-male DNA was detected between 10 pg to 150 pg/sample, corresponding to approximately 2-35 human cells. Both STR's autosomal and Y-STR amplification show that many of the samples consisted of a mixture of the victim and of least two men. One of these DNA profile showed match with other items found in the apartment like sperm in a condom and saliva on cigarette butt. Police investigations appeared to belong to a friend of the girl, met last night at the disco.
The other Y-STR profile was consistent with that of the boyfriend of the victim, who had found the body and had served first aid. The investigators believed that the friend knew at the disco was the author of the crime and arrested him, while the judge considered the other DNA profiles found on the victim's body due to random contact. This case demonstrates that the analyzes of touch DNA are useful for identifying individuals who have touched a body, but not sufficient for the resolution of investigations. It appears instead evident that it is real the possibility of introducing cognitive errors in investigations, where the sources of evidence are not adequately evaluated [1].

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358. A COMPARATIVE STUDY OF HUMAN AND ANIMAL HAIRS: MICROSCOPIC AND CYTOCHROME OXIDASE I (COI)

SPECIES IDENTIFICATION

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Human and animal hairs have been used in forensic investigations for over a century. Hair is stable under adverse natural conditions, hence, hairs are recovered along at the crime scene. Here, it is necessary to determine whether the hair is human or not. Morphological and genetics characteristics are useful to differentiate human from animal hairs.
However DNA technologies are time and cost consuming and often provide an incomplete genetic profile or inconclusive results. Therefore, a first step in the analytical process is the microscopic identification and comparison of human and animal hairs, in order to identify the organism to which the hair belongs.
The analysis of the hair morphology allows to achieve relevant information, primarily for the discrimination between human and animal hair, but can also allow to define the breed, the area of the body from which it comes, the growth phase, etc.
The present study is carried out to present a photomicrograph collection of hairs characteristics to use for a primary taxonomic distinction and the results regarding genetic testing through Cytochrome *c* Oxidase I mitochondrial gene (COI) analysis.
Preliminary results demonstrate that both the microscopic and genetic analysis of hairs are useful in forensic investigations.

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359. A PRELIMINARY STUDY ON THE NGS ANALYSIS OF THE MICROBIAL COMMUNITY STRUCTURE IN THE DROWNED PIG'S SKIN

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Estimation of time since death in drowning cases is very important in forensic investigation. Several methods to determine postmortem submersion interval (PMSI) in drowning cases have been suggested. In this study, we investigated the microbial community structure in the drowned pig's skin by NGS analysis in order to evaluate the possible application of this approach in determination of PMSI. Skin samples from pig drowned in lake were collected daily for six weeks from 25 June 2016 to 3 August 2016. 18S rDNA V1-V2 variable region was amplified and sequenced using the Illumina Miseq. NGS analysis of 36 samples showed that the microbial community structures were grouped by an interval of one-week. In further analysis on the relative frequency of the five most abundant genera, some

recognizable pattern of their increase and decrease at one-week interval was observed. The current preliminary results suggest that this approach using NGS analysis on the microbial community structure might be applied to estimate PMSI in drowning cases of forensic investigation, if further extended experiments are carried out.

360. A VALIDATION STUDY FOR THE EXTRACTION OF DNA FROM FORENSIC SAMPLES USING MAGCORE HF 16 PLUS AUTOMATED NUCLEIC ACID EXTRACTOR

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DNA extraction from forensic samples on MagCore HF 16 Plus automated nucleic acid extractor (RBC Bioscience, Taiwan) was done using MagCore® Genomic DNA Forensic Kit. The whole purification process is fully automated and requires no pre-treatment. The scope of forensic samples comprised standard reference samples (mouth swabs taken on cotton and ryon swabs (Copan, Italy)), blood, saliva and sperm on storage cards, cigarette ends, hair samples, chewing gum, and touch samples on lifting tapes. Automated extraction was run in pentaplicates, comparative manual extraction was run in duplicate using ZR Genomic DNA™-Tissue MiniPrep kit (Zymo Research, USA). The quality and quantity of extracted DNA from both automated and manual extractions was assessed using Quantifiler® HP DNA Quantification Kit (Thermo Fisher Scientific, USA). Selected extracts were amplified using AmpFLSTR® NGM SElect™ PCR Amplification Kit (Thermo Fisher Scientific, USA) and results compared. The data obtained demonstrate the versatility of the automated nucleic acid extractor for a broad spectra of forensic samples.

361. ANALYSIS OF HUMAN DNA PRESENT IN THE DIGESTIVE TRACT OF CULEX QUINQUEFASCIATUS AND AEDES AEGYPTI MOSQUITOES FOR POSSIBLE FORENSIC APPLICATION

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Forensic Entomogenetics deals with the application of entomology allied to molecular biology techniques, being a tool for obtaining evidence that can asist in the solution of crimes. An application of forensic entomogenetics is the identification of the genetic profile of an individual by DNA analysis of the digestive tract of a mosquito that has stung it and can be used in cases of kidnapping and captivity. The main objective of this work is to expose a group of mosquitoes of the genus Culex and Aedes to human blood (donor) and observe how long it is possible to detect traces of the human DNA that serves to identify them. For this, two groups were defined, one fed with human blood and the other fed with sugar solution (control). For a period of up to 120 hours, groups of 20 individuals are separated at different times for DNA extraction using the QiAmp DNA Investigator kit (Qiagen). In a preliminary analysis, the

concentration of human DNA determined by real-time PCR (qPCR), obtained from a mosquito, after thirty minutes of its feeding, was found to have resulted in 116.6 pg/μL. PCR amplification of this material with human mitochondrial marker was positive. The viability of the tested process for obtaining DNA and amplification of specific marker for human from mosquito was confirmed. It is hoped to determine the viability of the process tested for the detection of insect human blood over 120 hours, thus contributing to investigative actions within the forensic sciences.

362. ANALYSIS OF MARINE MICROORGANISMS EXTRACTED FROM THE INTERNAL ORGANS OF A DROWNED PIG

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Many studies have been done to investigate the cause of death by examining the anatomical characteristics of the drowned body. These include methods of analyzing diatoms, examining the unique features found in a drowned body and estimating the time of death based on the degree of decomposition in water.
NGS(Next Generation Sequencing) has advanced for many years and recently its application expanded beyond diagnostic medicine to forensic science. NGS is also suitable for analyzing marine specimens in which diverse organism's populations are clustered. Therefore, this study compared marine specimens to the organs of a drowned animal by using NGS to determine the cause of death and to estimate the location.
Marine specimens were collected by using the Plankton net and Grab from Pyeongtaek port and Gunsan port. And six organs including the lung were extracted from a pig(IRB: SNU-160629-12) after it had been drowned for a certain period of time. Then, the 18s ribosomal RNA gene was targeted, extracted and amplified, with which NGS was conducted. After that, we examined the similarities between marine microorganisms found marine specimens and organs extracted from the pig.
Results of this study demonstrate that the major species analyzed in those ports have many differences, whereas marine microorganisms found in marine specimens were identical with those found in the organs of the drowned pig. results suggest that the location of drowning could be estimated as well as the cause of the death could be determined by analyzing the drowned body by using NGS.

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363. APTAMER-BASED SENSOR FOR THE IDENTIFICATION OF FEMALE-SPECIFIC BLOOD STAIN

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Blood stain evidence obtained from violent crime scene plays a very important role in providing a definitive clue to resolve the case through DNA genotyping in forensic field. However, in most case of the crime scene evidence, it was often the case that the suspect and the victim's blood were mixed or dispersed in various parts of the evidence. In this case, it caused the mass sampling of the collected samples in the crime scene evidence and consequently makes it difficult to DNA genotyping. Herein, we reported the activatable aptamer sensor targeting 17β-estradiol to detect the female specific blood specimens as forensic methods. This aptamer sensor (0.2 nmoles/$\mu\ell$) could easily distinguish the female specific blood using variable light source (495 nm) in crime scene blood samples, but not the male blood. In particular, it was able to detect the young female (10s ~ 40s aged) blood in whole female blood samples, but not the older female (50s or more aged). Genomic DNA was extracted from female blood samples collected in this way, and DNA profiling data was obtained by quantification and short tandem repeats (STR) genotyping analysis. As a result, we confirmed that fluorescence interference due to this aptamer sensor did not occur. Therefore, application of this aptamer sensor may help to collect the samples selectively at the crime scene evidence, and consequently the DNA genotyping can be easily performed.

364. ASSESMENT OF METHOD FOR BUCCAL SWAB SAMPLES PRESERVATION IN EXTREME ENVIRONMENTAL CONDITIONS FOR POPULATION GENETICS AND FORENSIC PURPOSES

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The lack of infrastructure that guarantees the physical-chemical integrity of DNA and the procedures for sample collection in forensic caseworks at extreme environments (tropical rain forest with humidity 86-96% and temperatures from 36-46°C) are a huge problem for preservation of biological samples. Swab samples must be dried and maintained at low temperatures as soon as possible after their collection. Nevertheless, environmental conditions in those zones prevent proper drying, turning samples into a broth culture of microorganisms. Thus, a cheap and accessible protocol for sample preservation, that prolongs DNA quality, is essential for forensic and population genetics sampling. In this research, a preserving method of buccal swab samples, collected under extreme conditions, has been evaluated using TNE-Ethanol-Buffer. This poorly known method inactivates nucleases and prevents the development of microorganisms. The huge advantage of this little diffused method is that the swab sample does not require a drying or freezing process after its collection. Dried buccal swabs and TNE-Ethanol Buffer swabs from 10 volunteers were evaluated under different temperatures (-20°C / 4°C / 37°C / RT) and time frames (0-4-8 days). DNA extraction was obtained by Phenol-Chloroform-Isoamyl-alcohol method. The DNA quality and concentration were evaluated by spectrophotometry (90-285 ug/µl; 260/280=±1,9). Agarose electrophoresis and Identifiler PCR amplification showed high quality DNA, and full genetic profiles were obtained from samples conserved in TNE-Ethanol Buffer. DNA

preservation rate was always higher in swab samples preserved in the buffer while the dried swabs contained less DNA over time.
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1. Aidar M, and Line SA: Simple and Cost-Effective Protocol for DNA isolation from buccal ephitelial cells. Brazilian Dental Journal. 2007; 18: 148-152

365. COMPARATIVE STUDY BETWEEN A DIRECT DNA QUANTIFICATION METHODOLOGY AND THE STANDARDIZED METHODOLOGY IN THE FORENSIC WORKFLOW

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The acquisition of the genetic profile from a biological sample can be performed through the study of nuclear DNA using STR markers. In a forensic laboratory, the workflow involves the selection of suspicious samples, the DNA extraction/purification, quantification and subsequent STR amplification. Being the PCR reaction strongly affected by the DNA amount added to the reaction, it is crucial to know the DNA quantity and quality present in a sample, in order to add the optimal input of DNA to the PCR reaction. Direct quantification consists in a real-time PCR method in which one punch of 0.5 mm from a biological sample is placed in the reaction well without having previously been subjected to an extraction step. Therefore, the main objective of this study was to evaluate the feasibility of implementing a methodology of direct quantification of DNA in the routine workflow of a forensic laboratory. For this, blood, saliva and semen were placed on various FTA® cards, and were directly quantified. Every directly quantified sample had a replica subjected to DNA extraction before quantification. The DNA quantification was performed with the Quantifiler® Trio DNA Quantification Kit and amplified with the GlobalFiler® PCR Amplification Kit. From the results of the direct quantification it was possible to determine the number of punchs necessary to obtain an optimal input to the direct amplification reaction of the same samples. Therefore, the combination of direct quantification and amplification allows to obtain a simpler workflow with faster results and less costly procedures.

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366. DECREASE DNA CONTAMINATION IN THE LABORATORIES

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It is crucial to avoid laboratory contamination. Hypoclorite is known to be very efficient in removing any traces of DNA and this chemical is therefore commonly used for cleaning in many DNA laboratories. However, hypoclorite can produce poisonous chlorine gases when it is reacting with acidic solutions. In this study, we tested alternative approaches to remove DNA from hard laboratory surfaces. We contaminated clean surfaces with four different concentrations of massively parallel sequencing libraries. The DNA was dried and left for 45 minutes and for 24 hours, respectively, before any treatment. The surfaces were cleaned with six different methods: water, 96% ethanol, water followed by 96% ethanol, 10% hypoclorite, 3% hypoclorite, and no treatment. Subsequently, the surfaces were swabbed using cotton swabs. DNA was extracted from the swabs and the DNA concentrations were determined by real-time PCR. The results showed that leaving the DNA traces for 24 hours decreased the amount of amplifiable DNA with 4 times. Similar, cleaning the surface with 96 % ethanol also reduced the amount of amplifiable DNA 4 times. Cleaning with water and water followed by 96% ethanol reduced the amount of amplifiable DNA 100 times, whereas cleaning with hypoclorite removed all traces of amplifiable DNA. This indicated that the 'mechanical' cleaning was efficient but cleaning with hypochlorite was superior. In conclusion, it is recommended to clean laboratory surfaces with 3% hypochlorite in order to eliminate laboratory contamination.

367. DEGRADATION OF AIF MRNA IN RAT HEART TISSUE FOR ESTIMATING POSTMORTEM INTERVAL (PMI)

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Estimating time of death in forensic is particularly important, though a range of time can be determined by traditional methods. However more accurate time of death is needed in forensic case work. Rate of mRNA degradation is considered as a mean to increase accuracy of postmortem interval (PMI) estimation. Different mRNAs result in different rate of degradation since time of death. Besides, our previous results proposed that DNA was suitable as normalized gene. So in this study we determined apoptosis inducing factor (AIF) mRNA degradation of Caspase-3 DNA to establish a mathematical model with 48 hours PMI to estimate time of death. In addition, corpses of mice were placed in 37 degrees to simulate summer environment of Chengdu and heart tissue was obtained in each time point. RNA and DNA were co-extracted with Bioteke co-extraction kits followed by expression level detection with RT-qPCR of MiScript SYBR Green PCR Kit. The results showed that in 48 hours PMI AIF was slowly degraded. What's more, AIF represented a faster degradation process and a good liner relationship within 14-20 hours PMI. Therefore, we suggest degradation of AIF is a reliable and accurate method to estimate time of death in14-20 hours PMI.

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368. DETECTION OF DNA PRESENT ON TAPE-LIFTS USING FLUORESCENT IN SITU DETECTION

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Tape-lifts can be used for the removal of evidence from items of interest without damaging the substrate. In addition to loosely adhering material such as hairs and fibres, tape-lifts can be used to remove latent cellular material such as that transferred to clothing when worn or in close proximity to the mouth. Common forensic practice is to examine the entire tape-lift in a non DNA targeted approach. The research presented illustrates the use of DNA binding dyes as a DNA-targeted screening tool for tape-lifts. The individual cells can be visualised allowing sections where cells are present on the tape to be removed for subsequent DNA typing. Two different dyes, EvaGreen™ and Diamond™ Dye, were used to stain tape from which full single source DNA profiles were generated that matched the expected control. Direct amplification was undertaken from tape-lifts of samples where biological material had been deposited over 12 months previously on glass slides from which mixed profiles were obtained. The process demonstrated outlines an effective means to visualise the presence of cellular material from which STR profiles can be generated allowing for a targeted approach to be performed.

369. DEVELOPMENT OF A TRIAGING PROCESS USING QUANTIFILER HP TO ASSIST IN CHOOSING AN APPROPRIATE DOWNSTREAM AMPLIFICATION METHOD FOR POST MORTEM IDENTIFICATION OF HUMAN REMAINS

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The Victorian Institute of Forensic Medicine (VIFM) routinely receives post mortem (PM) samples for the purposes of scientific identification that yield DNA extracts with low concentration, degraded DNA or a combination of both. These extracts often yield incomplete DNA profiles that require additional work to produce a more complete profile. The VIFM uses various methods such as multiple amplifications, mini-STRs and mitochondrial DNA analysis when a reportable result is not obtained in the first instance. However, any additional work results in increased time and costs for the laboratory. The aim of this project is to develop a guide that predicts the completeness of profile with downstream amplification methods based on the concentration and degradation index of a DNA extract.
A matrix of samples with different levels of degradation and concentrations was generated. Whole blood samples were extracted and subjected to set time intervals of enzymatic degradation followed by serial dilution. DNA concentration and degradation index were obtained using Quantifiler® HP followed by

amplification using the GlobalFiler™ and MiniFiler™ Kits and subsequent analysis using GeneMapper® *ID-X*. Each profile was assessed for completeness, stochastic effects and loss of larger loci due to degradation. Using data from a single amplification at each degradation index and concentration combination in the matrix, a method was developed to predict completeness of profile when additional GlobalFiler™ amplifications are to be performed. This data was used to produce a guide that suggests the best downstream amplification method for a sample based on its concentration and degradation index.

370. DNA ANALYSIS OF DRUG-RELATED CRIMES OCCURRED IN GYEONGSANGNAM-DO AND BUSAN IN 2016

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Several types of evidences are commonly used in forensic science for the purpose of DNA analysis. The detection of DNA in drug-related crime can be a powerful evidence to prove a defendant's guilt. Recently, drugs in Korea are perceived as a serious social problem because drug smuggling from abroad and drug trafficking by organized gangs is continuously increasing. The recidivism rate in drug-related crime which is increasing every year is 37.6 percent in 2016. So, searching of established forensic DNA databases have important role to arrest suspect of drug-related crime. Illicit drug abuse in Gyeongsangnam-do and Busan occurs at high levels because of a relatively large abuser population. So, evidences such as syringe, hair and urine from drug-related crimes occurred in Gyeongsangnam-do and Busan submitted to Busan institute of National Forensic Service to investigate DNA are much higher than other local institute of National Forensic Service. In 2016, 5,273 subjects from crime scenes occurred in Gyeongsangnam-do and Busan are submitted to Busan institute of National Forensic Serevise to investigate DNA. Among them, 226 subjects (4.3%), 1,254 evidences (3.2%) were from drug-related crime. When compared to 2015, the number of drug-related cases and evidences has increased by 56% and 35%, respectively. Breaking down the evidences by the type, 90% were syringe while 1.3% and 0.4% were hair and urine, respectively. DNA was successfully isolated from 905 (80%) out of 1131 syringes. The DNA isolated from 561 (50%) syringes is matched with target persons (suspect, convict and arrested suspect) while 225 are failed to isolate DNA.

371. DNA EXAMINATION OF SEXUAL ASSAULT WITH MULTIPLE ASSAILANTS

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The incident occurred in approximately midnight on January 2016. The victim returned home by a motorcycle alone and was attacked by 2 assailants. The assailants jostled the victim's motorcycle, and one of them escaped with the vehicle. The other assailant forced the victim to ride with him and drove into the deserted field. The assailants committed sexual assault, and raped the victim before they escaped. The victim filed a report with the police and was sent for physical examination and vaginal swab to collect the suspects DNA. Subsequently, the police were able to arrest 2 suspects and their

buccal swab was sent to the laboratory for examination.The buccal swabs were extracted using Chelex method,16 loci of STRs were amplified using Investigator IDplex Plus Kit, the analysis revealed full STRs DNA profiles. The vaginal swab was extracted by differential extraction method using QIAamp DNA Investigator Kit. The analysis also revealed full female DNA profile from the epithelial cell, mixture DNA profiles of two male were found from the sperm sample, major peak and minor peak were separated and revealed partial DNA profile (14 loci) of STRs from major peak, the results demonstrated a match to the suspect number 1 with posterior probability 99.99999999%. Another partial DNA profile(8 loci) also demonstrated a match to the suspect number 2 with posterior probability 99.99997985%. The evidence was able to lead to the arrest of the assailants and proceed according to the law.

372. ESTABLISHMENT OF BASIC FORENSIC ENTOMOLOGICAL DATA FOR FORENSIC APPLICATION

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The estimation of minimum postmortem interval (PMImin) using entomological evidence is known as one of the most accurate methods, even when the body is badly decayed. For the establishment of basic forensic entomological data and the development of a software for the estimation of PMImin in Korea, a research consortium was constructed and consisted of 1) the study on Korean nercophagous entomofauna according to natural environment and their DNA Barcoding, 2) the construction of ecological database related to Korean necrophagous insects and forensic entomology, 3) the study on statistical models for forensic entomology and meteorology, 4) the estimation of PMImin based on developmental gene expression clock of necrophagous fly, and 5) a consultation on programs by a bioinformatics company. As results of initial research, in the mid-north area of Korean peninsula, 20 families 37 species were identified from decomposed piglet experiments and fly traps. In the mid and southern area, 35 families 95 species were investigated with coding of insect samples for fast biodiversity survey. By correlation studies on several factors related to estimation of air temperature, highly correlated variables were selected and a preceding estimation algorithm was established. A genome project for *Sarcophaga peregrina*, contig assembly and annotation for 4 fly species, and the securement of growth cycle specimens were performed. After these studies, it is expected that investigation timeline in scene will be shortened and the capacity of investigation on cases of extremely decomposed bodies will be strengthened.

374. FLIPTUBE® TECHNOLOGY PROMOTES CLEAN MANIPULATION OF FORENSIC SAMPLES ON AUTOMATED ROBOTIC WORKSTATIONS

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Forensic examiners often manipulate evidentiary materials in microcentrifuge tubes. Open tubes, and the need to have multiple tubes open at the same time, present an important opportunity for sample contamination. Contamination risk is made more acute during automated processing of forensic samples, due to the need to decap and rack multiple samples onto the robotic platform at one time and leave them uncapped during entire processes. Thus, sample-to-sample and technician-to-sample manipulations are real concerns as long as tubes remain open, and the automated opening and closing of conventional microcentrifuge tubes is not possible. FlipTubes are the first of their kind, and were specifically developed by Hamilton Robotics automate the opening and closing of microcentrifuge tubes in order to increase walk-away time and decrease the risk of sample contamination. FlipTubes feature a precisely sealing lid to minimize sample evaporation, optical control with graduated lines marked at 0.1, 0.5, 1.0, and 1.5 mL, and a large frosted lid and surface on the side for easy marking. FlipTubes are human DNA free, RNase- and DNase-free, ATP-free, and non-pyrogenic. In response to both real and perceived court concerns regarding evidence sample integrity, the Broward County Sheriff's Office Crime Laboratory has adopted FlipTube® technology to promote the clean manipulation of forensic samples on its automated robotic workstations. In the first instance, FlipTubes have been incorporated into the quantification through amplification steps of the pre-PCR DNA typing workflow. This workflow, with the incorporation of FlipTube® technology, is currently being validated for use in forensic casework.

375. FORENSIC DROWNING SITE IDENTIFICATION EMPLOYING A MIXED PYROSEQUENCING PROFILE OF DIATOM DNA-BARCODE

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On account of the peak of pyrosequencing contains quantitative information, it has been used for forensic multi-allelic SNP assessment in pooled DNA. In our preliminary study, we constructed an 18S V7 pyrosequencing system that can be used for decoding the mixed diatom DNA profile and identifying the water sources where the diatoms came from. In the present study, we constructed a drowning model to verify the effectiveness of the system when applied to forensic identification of the drowning site. Five identified diatoms were cultured and mixed together as a simulated drowning fluid. Then we established a diatom drowned animal model by trickling "the drowning fluid" into the rabbit trachea till respiration ceased. To recover the entire diatoms inhaled into the lung, trypsin and collagenase were used to digest the connective tissue and secretions in lung. After eliminating the rabbits DNA with DNase I, the diatomic DNA was extracted with Chelex-100. The 18S V7 target region was amplified and pyrosequenced. The mixed pyrosequencing profile was decoded with the AdvISER-M-PYRO program of R-Project (https://www.r-project.org/). Five diatoms aspirated into lungs were identified successfully. The results indicate that the pyrosequencing is a potentially useful method for inferring the drowning site.

376. FORENSIC IDENTIFICATION OF SOIL USING METAPOPULATIONAL ANALYSIS OF THE BACTERIAL IV 16SRNA REGION

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Forensic analysis of soil uses elemental analysis, macro- and microscopic analysis of soil and botany-based analysis. We present the results of initial analyses of 70 soil samples in terms of the composition of soil bacteria population for use in forensic analysis of soil.
The studies were employed due to the discovery of two young female bodies in an aquatic tank. The soil samples from shoe soles of men suspected of murder were secured. Soil samples from crime scene and similar areas and the residences of suspects and victims were subjected to studies.
DNA was extracted from 500 mg of soil sample using GenomicMiniAXSoil kit (A&A Biotechnology). Region IV of bacterial 16S RNA gene was amplified using universal primers 515F and 806R. The libraries were constructed from amplicons using NEBNext® DNA Library Prep kit (New England Biolabs UK). Sequencing was conducted on an Illumina MiSeq using paired-end (2x250) MiSeq Reagent Kits v2 (Illumina, USA). The sequencing data was processed using CLC Genomic Workbench 8.5 and CLC Microbial Genomics Module 1.2. (Qiagen, USA).
Metapopulational comparisons allow for an unequivocal identification of soil samples. Based on the obtained results it should be concluded that the described method may be useful for forensic identification of soil. The potential issues for the proposed study methods are mixed soils, stacked from different areas and in different time, which may notably limit the applicability of the method, which does not search for indicator species and the identification is based on a qualitative-quantitative criterion.

377. GENOME-WIDE COPY NUMBER VARIATION ANALYSIS IN MONOZYGOTIC TWINS

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Monozygotic (MZ) twins are genetically nearly identical as they are derived from the same zygote, which makes it impossible to distinguish one from another by forensic short tandem repeat (STR) profiling or single nucleotide polymorphism typing. However, increasing evidence indicates that copy number variations (CNV) exist within pairs of MZ twins, especially when they are phenotypically discordant. These results suggested that a genome-wide comparison of CNV profiles between MZ twins might provide an insight into forensic discrimination of MZ twins. Here, using the high-resolution Agilent SurePrint G3 Human CGH microarray we compared the DNA CNV profiles in blood samples collected from 12 pairs of MZ twins. Following an effective and unbiased approach, we detected 1345 CNV loci in pairs of MZ twins. Among them, 31 were shared in at least half of the samples. This pilot study suggested the potential usability of CNVs in individual identification of MZ twins.

378. INFLUENCE OF INSECT ACTIVITY ON THE DYNAMIC CHANGE OF POSTMORTEM MICROBIAL COMMUNITIES

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Dynamic change of microbial communities in a clock-like manner provides a promising method for postmortem interval (PMI) estimation. Previous studies have suggested that sarcosaphagous insects and their carried microorganisms were potential competitors of postmortem microbial communities. But how insect activity affect microbial communities remains poorly known in carrion decomposition systems. In present study, rabbit carcasses were treated as insect exclusion and access groups. Bacterial were sampled from buccal cavity and rectum of rabbits at various period of time after death and analyzed by high throughput metagenomic technology. Our results showed that the time length of cadaver decomposition in insect exclusion group was longer than that of insect access group. But the pattern and time cost of microorganism succession was nearly similar between the two groups, though the latter decomposed more rapid than the former. Meanwhile, the activity of insects had no significant impact on the species diversity of bacterial communities during decomposition. But the abundance of some bacterial taxa could be disturbed by the arrival of insects. In addition, significant variation in species diversity and relative abundance of bacterial families between buccal cavity and rectum samples were detected. Our research indicated that the succession of microbial community structures was a relatively stable continuous process even under insect disturbance. This result further proved the reliable value of microbial evidence in forensic investigation.

379. MODIFIED DIFFERENTIAL DNA EXTRACTION TO REDUCE PROCESSING TIME OF SEXUAL ASSAULT EXHIBITS

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Differential extraction using the conventional phenol/chloroform method is widely used for the separation of sperm and female epithelial cells in sexual assault cases. This method, however, is laborious and requires multiple manual tube-to-tube transfer steps. To make the method more efficient, the laboratory incorporated semi-automation in the form of Maxwell DNA extraction. Briefly, the samples were first incubated in buffer and the protein supernatant and cell pellet were separated following centrifugation. The supernatant was retained for seminal protein assays (such as Seratec PSA and RSID Semenogelin). The cell pellet was then treated with detergent and proteinase to lyse the female epithelial cells and facilitate separation into female epithelial and male sperm DNA fractions. DNA extraction was then performed on both fractions on the Promega Maxwell® 16 instrument. To assess the sensitivity of this modified method, intimate swabs and sexual assault exhibits were stained with various dilutions of human semen ranging from 1:10 to 1:1000. This modified method was compared with the conventional phenol/chloroform method to assess DNA yield and separation of the female epithelial and male sperm DNA fractions. This modified method uses a shorter time with reduced human intervention which, also reduces the risk of contamination or error. This modified method is also consistent with the "Direct to DNA" approach highlighted in the 2016 SWGDAM "Recommendations for the efficient DNA processing of sexual assault evidence kits" as it allows for concurrent testing of both protein assays and DNA.

380. PCR-BASED TESTS FOR FORENSIC DETECTION OF FECES; USE OF BACTEROIDES SPECIES AS INDICATORS OF FECAL CONTAMINATION

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Currently there are four body fluids that can be detected in the forensic DNA laboratory: blood, semen, saliva and urine. *Homo sapiens* produce a variety of other body substances (sweat, tears, ear wax, vaginal secretions, etc.) for which no reliable stain identification tests are available. In response to the needs of a large urban jail, our laboratory has developed a forensically suitable, PCR-based method for the identification of feces on items of evidence. The test is human centric (i.e., not unique for human) and based on extensive published research in fecal contamination of water supplies. These studies have shown that the 16S rRNA gene of *Bacteroides dorei (B. dorei)* is an excellent marker for aggregate environmental human fecal contamination; however, *B. dorei* is not universally detectable in all individuals. For a forensic application to identify individual human donors, we therefore introduced a second PCR assay which detects multiple *Bacteroides* species reported to account for 30 - 40% of total fecal bacteria. This second test has broader animal specificity, but captures those human individuals whose *B. dorei* titer is below the limit of detection of the first assay. The final, validated workflow incorporates a commercial kit for the purification of DNA, two amplification reactions with dye-labeled PCR primers and internal amplification controls, and analysis of the amplification reactions by capillary electrophoresis. This method provides a simple, robust and to our knowledge, novel forensic test for the identification of human feces.

381. POSTMORTEM INTERVAL (PMI) DETERMINATION BY PROFILING OF HAF MRNA DEGRADATION USING RT-QPCR

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Postmortem interval (PMI) determination, helping exclude or confirm suspects, plays a key role in forensic cases. Recent years, degradation of mRNA has shown potentiality to determine time of death and be allowed to obtain a considerable time since death. In our study, we aimed to find an mRNA marker to assess PMI with accuracy. We performed hypoxia associated factor (HAF) mRNA degradation in 48 hours after death within 29 time points in rat brain to seek a more precise time of PMI determination. Caspase-3 DNA was carried out as normalization of HAF mRNA degradation. Nucleic acids were extracted by commercial kits and relative quantitive PCR was used to detect expression level of HAF mRNA and Caspase-3 DNA. Through analyzed profiling of HAF mRNA degradation, we acquired a statistical model between 48 hours PMI and mRNA degradation. Then we found that abundance of 105bp HAF mRNA fragment was increased in 48 hours. Interestingly, in the first 4 hours after death, PMI was well correlated with HAF mRNA degradation in rat brain. We believe these results indicate that HAF mRNA was a suitable marker for PMI determination in early time since death and the statistical model of it can become a useful tool in forensic practice.

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382. PRESUMPTIVE TESTS: A SUBSTITUTE FOR BENZIDINE IN BLOOD SAMPLES RECOGNITION

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The nature of the sample in a forensic case is one of the most important factors, since it determines the posterior analysis, helping to define its identity (like blood *versus* semen). It could also aid to discard samples that would not be useful concerning a specific forensic case (for example, water *versus* saliva). A presumptive test is a qualitative analysis that allows to identify or confirm the presence of a substance in a sample. These determinations usually occur, after a chemical reaction, and a specific colour, agglutination or a solid formation is produced (1). A false positive is another substance reacting the same way, producing the expected result.
Associated with a possible delict, blood samples can provide essential information (2), which may help to solve a given case.
The aim of this work was to evaluate the most effective presumptive test (with fewer false-positives) when analysing products that could look and behave like blood during a forensic screening assay. In this study, nine different products were tested, like Betadine®, and four reagents were considered: Benzidine, O-toluidine, Leuchomalachite green and BlueStar® Forensic (BlueStar). Each product was tested with the reagents five times - mixed with human blood (3:1), with three different animal blood (3:1), and then unmixed. To verify if the physical support could influence the results, each assay was done in a cotton fabric, and in a glass support.
Our results indicated that Leuchomalachite green is the most suitable presumptive test, since it was the reagent with less false positives.

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383. THE EFFECTS OF METHAMPHETAMINE ON THE DEVELOPMENT OF BLOWFLY *ALDRICHINA GRAHAMI* (DIPTERA: CALLIPHORIDAE) AND CADAVERS BACTERIAL COMMUNITY

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Necrophagous fly species are commonly used as forensic tools for the estimation of the minimum postmortem interval (PMI-min). However, previous studies have shown that certain drug, would affect larval development, thus leading to a disturbed estimates of PMI-min. In this study, we investigated the effects of carcass contained methamphetamine on the development of blowfly *Aldrichina grahami* (Diptera: Calliphoridae). Larvae of *A. grahami* were exposed to rabbit corpses which injected with methamphetamine at different concentrations when alive (experimental group). And non-injected rabbits corpse used as control group. The larval lengths, weights, and developmental durations of each development stage were observed and recorded. Our preliminary data showed that there was no obvious difference between experimental group and control group in larval lengths and weights. But impressive difference could be detected at the time length of pupa stage. The eclosion of pupa in experimental group was significantly delayed when compared with control group, which possess potential forensic meaning in practical case. In addition, we sampled bacterial communities from buccal cavity and rectum of living rabbits, soon after died and various period of time after death. We sequenced 16S rRNA gene and found that significant difference existed in species and relative abundance of bacterial communities between experimental group and control group, which also has forensic significance.

384. THE USE OF WHATMAN™ FTA™ ELUTE TO SIMPLIFY STORAGE AND REANALYSIS OF EXTRACTED DNA FROM FORENSIC SAMPLES

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Forensic laboratories and their submitting agencies often have an obligation to maintain forensic DNA samples and extracts for use in future testing. Required by statute or laboratory policy, many of these samples are being stored as liquid extracts in freezers that are expensive and take up precious space within the laboratory. Whatman FTA Elute technology allows for long-term DNA preservation at room temperature combined with easy elution for future testing of individual samples. In contrast to classic FTA cards, the FTA Elute chemistry releases DNA into solution with a simple water and heat elution step. Eluted DNA can be used for many applications including STR analysis, sequencing, and real-time PCR.
Previous protocols for the FTA Elute technology focused on collection and storage of intact biological specimens. Optimized protocols for storage and elution of extracted DNA have now been developed to provide guidance to laboratories interested in room temperature storage of forensic DNA extracts. A general overview of the technology, protocols and experimental data supporting the use of FTA Elute for extracted DNA will be presented.

385. USING DNA FOR DRUG SMUGGLERS

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The evidence was sent to the laboratory for examination by Office of the Narcotics Control Board on May 2015. Many evidences were left in the forest by drug smugglers while they were chasing by officers. Those are backpacks, plastic bags, 5 snack packs, 2 spout pouchs and shirts. They were sampling by cotton swab and extracted by using QIAamp DNA Investigator Kit, 18 loci of STRs were amplified using PowerPlex®18D System Kit and 23 loci of Y-STR amplified using PowerPlex®Y23 System. Analysis found that STRs mixture in backpack and plastic bags. Partial profile in spout pouch number 2 and full profile in snack pack number 4. One month later the buccal swabs of suspect was send to the laboratory for examination and extracted by using Chelex method. 16 loci of STRs were amplified using Investigator IDplex Plus Kit and 23 loci of Y-STR amplified using PowerPlex®Y23 System. The results demonstrated a match of STRs and Y-STR suspect to full profile of snack pack number 4 with posterior probability 99.99999999%. The evidence sent the suspect to the prosecution.
Keyword: DNA, Drug smugglers

386. ALCOHOLS AS SOLUTION FOR DELAYING MICROBIAL DEGRADATION OF BIOLOGICAL EVIDENCE ON COTTON SWABS

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Poor storage of biological evidence on cotton swabs is known to lead to fungal and bacterial growth. However, there are often situations where it is inconvenient to dry the swabs without running the risk of contaminating the sample, or where only plastic bags are the best option for packaging material. This study aims to investigate the effectiveness of using 70% ethanol and 100% isopropanol to prevent or delay microbial growth on cotton swabs containing biological evidence and their effects on the subsequent STR analysis. For pre-evidence collection treatment, the alcohols were applied on cotton swabs as moistening agent prior to collection of dried saliva stains. The cotton swabs were then packaged immediately in sealable plastic bags and stored at room temperature (27-29ºC) for up to 7 days to simulate improper storage conditions. DNA was extracted and amplified using AmpFlSTR® Identifiler™ PCR Amplification kit to obtain STR profiles. The results showed no observable fungal growth on any of the swabs moistened with 70% ethanol and with 100% isopropanol in the study, and full STR profiles could be obtained from these swabs, while the growth was observed on swabs moistened with sterile deionized water after 5 days. The potential application for treatment on post-evidence collection where buccal swabs were sprayed with the alcohols was also examined. The outcome of this study could be used to suggest an alternative DNA evidence collection and storage method in remote areas where samples must be transported in non-ideal conditions to central labs for analysis.

387. ANALYSIS OF MIXTURE DNA IN MURDER CASE EVIDENCES

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A dead body of a female in her 50s was discovered along the hiking path in Muhak Mountain (Korea), with severe head wounds and signs of strangulation. There was no witness, and the CCTV camera had not been installed at that location, either.
Using forensic light source equipment, we collected 29 minute traces off the victim's gloves. Of those, mixture DNA that contains the victim's along with 1 male's was found in 5 touched traces. We performed analysis using the AmpF*l*STR® Identifiler® Plus PCR Amplification Kit(Applied BioSystems), the Yfiler® Plus PCR Amplification Kit(Applied BioSystems), PowerPlex® Fusion System(Promega) and the PowerPlex® Y23 System(Promega) to improve the accuracy of the data. To deduce a single DNA trace, we reflected amplified peak height values in each STR marker while excluding the known victim's DNA from the mixture profiles. Combining the outcomes of analyses led to single DNA for 1 male, except for the victim's DNA. However, the DNA of the male found on the victim's gloves did not match the person named the prime suspect by the police.
With an unknown male DNA profile, a search was performed from the offender's DNA-DB. A match was found between the unknown male DNA and an offender in the DNA-DB. The real criminal in prison confessed to the crime after being shown all of the DNA evidence found during the prosecutors' investigation. All charges were dropped against the innocent citizen who was falsely arrested.

388. CASE REPORT: MALE PHENOTYPE WITH INCOMPLETE Y CHROMOSOME AND X CHROMOSOME DOUBLE DOSAGE

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Sex typing constitutes a crucial process in forensic areas as criminalistics and filiation. Routinely, most of the autosomal Identification STRs Kits include the amelogenin gene for this purpose. In criminology, the sex identification plays a preponderant role, since it gives an idea to the prosecutor´s office to search for a suspect with male or female phenotype, as well as in sexual offenses it is essential to determine the presence of male component in a vaginal swab or related evidence.
This work reports a 53 years old man, apparently healthy, excluded as father in a case derived from paternity testing service. Molecular analysis was performed, according to the manufacturer's recommendations. PowerPlex®16 system and Identifiler® showed lack of amelogenin gene in Y chromosome; YFiler® profile showed only four loci DYS393, DYS456, DYS458 and DYS19, other markers were absent. The X-STRs by Decaplex kit[1] and Investigator® Argus-X12 showed heterozygotes genotypes in almost all loci suggesting the presence of two X chromosomes (as female profile). These findings suggest that q arm and part of p arm of Y chromosome was deleted, but this did not explain the lack of amelogenin gene because of the presence of DYS456 and DYS458 markers. Double dosage of X chromosome could be explained by two hypotheses: first, Y short arm with deletions is translocated to X chromosome due to paternal non-disjunction during meiosis; second the subject has the 47,XXY condition and same deletion of Y chromosome. Other analyses are required to improve the genetic characterization of this case.

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389. CIRCULATORY MICRORNA IN ACUTE MYOCARDIAL INFARCTION: A CANDIDATE BIOMARKER FOR FORENSIC INVESTIGATION

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MicroRNAs (miRNAs) are small non-coding RNAs regulated a gene expression which associated to many diseases. As they contribute to diverse biological process and respond to various kinds of cellular stress, their utility as diagnostic biomarkers have recently been explored. miRNAs have been detected in various type of human tissues and were used as novel biomarkers in clinical investigation for example; ischemic stroke, acute myocardial infarction (AMI) and several forms of cancer cells. Recently, circulatory miRNA was proved that It could be a potential biomarker to determine a cause of death. In order to verify the ability of miRNA as a diagnosis tool, 3 candidate miRNAs (miR-133a, miR-208b and miR-499) associated to an early response of cardiac injury were selected. Twenty case-control cohorts using post-mortem blood samples were studied for miRNAs expression and post-mortem stability. The expression level of miR-499 was significantly increased in AMI groups compared to controls. The post-mortem blood was in vitro tested during 12-18 hours which reveal a persistent of miRNAs in early decomposed environment.

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390. COMPARISON OF FLUOROMETRIC AND REAL-TIME PCR QUANTIFICATION OF DNA EXTRACTED FROM FORMALIN FIXED TISSUE

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Formalin-fixed, paraffin-embedded (FFPE) tissue provides an alternative source of genetic material for medical diagnosis and forensic casework. There are several protocols for the extraction of genomic DNA from FFPE tissue samples that try to overcome the cross-linking and fragmentation of nucleic

acids caused by formaldehyde. Therefore, the assessment of the quality and quantity of DNA extracted from formaldehyd fixed tissue is determining factor for downstream analysis. The following three methods are frequently used to quantify DNA: 1) UV absorbance; 2) fluorometric assays; and 3) relative quantification of a particular DNA sequence based on real-time PCR.
The objective of this research was to compare the quantity of extracted DNA from liver tissue when preserved in different solutions of formaldehyde with different duration of fixation and to decide which method is the most suited for the quantification. We compared the measurement of the DNA concentration by fluorometric assay (BioSpectrometer (Eppendorf, Germany) in conjunction with Qubit® dsDNA HS Assay Kit (Invitrogen, USA)) and qPCR-based assay (primers targeting sequence of ALU transposable element with two different amplicon size 63 bp and 229 bp). The spectrophotometric method was not applied due to its low sensitivity with measurement of low DNA concentration. The fragmentation of extracted DNA from fixed liver tissue was visualized on Agilent 2100 Bioanalyzer with High Sensitivity DNA Analysis Kits (Agilent Technologies, USA). We also calculated the degradation index (DI).

391. GENETIC SCREENING OF SUDDEN CARDIAC DEATH GENES IN FFPE SAMPLES USING HALOPLEX HS

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Sudden unexpected death in a young individual can be the first clinical manifestation of a genetic cardiac disease. Screening of genetic variants in a forensic setting has been possible for years using fresh blood samples. However, it is not uncommon that screening of old cases were only FFPE material is available is required. In this project we therefore aimed at evaluating Haloplex Target Enrichment HS of 84 cardiac genes designed for FFPE samples. We optimized the extraction, amount of DNA input and library preparation. DNA from two platinum genomes and nine cases where DNA from fresh tissue and FFPE (2-4 years) material were available. Haloplex HS and MiSeq sequencing was used. The results show that the amount of DNA needs adjustment based on fragmentation patterns. For FFPE samples with a middle fragmentation average (1500-3300bp peak) a median coverage of 1074X, average read length was 97bp and a median of 99% of the bases in the target region was covered by >=20X. For the FFPE samples with a high fragmentation (290-650bp peak) a median coverage of 754X, average read length was 82bp and a median of 90% of the bases in the target region was covered by >=20X. Of the 1549 filtered variants found using DNA from fresh tissue, 52 was not covered in the FFPE samples (34 of in one sample) and six false positive/negative variants were identified. In conclusion, individualizing the protocol based on the sample quality results in Haloplex HS method suitable for screening of FFPE samples.

392. OPTIMISATION OF DNA RECOVERY AND STORAGE FOR URINE AUTHENTICATION

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Denial of ownership of urine samples submitted by athletes for doping control has led to an increasing need to incorporate DNA analysis into the test protocols. As there are often large amounts of samples to process, a DNA extraction method suitable for urine samples that is quick, reliable, and economical is desired. FTA® cards have been employed extensively for preparing and preserving genetic materials at room temperature for long periods of time. This study explored the possibility of using FTA® card as a means to store urinary DNA. The sample preparation steps for harvesting cells from urine, such as centrifugal speed and washing, which could affect the DNA yields, were optimised. The performance of FTA® card was evaluated against two other DNA recovery techniques: Chelex® extraction and Wizard® SV Genomic DNA Purification Kit, for the lowest amount of urine sample required to generate a complete DNA profile using AmpFISTR® Identifiler™ PCR Amplification kit. Preliminary results from a 300-bp single STR locus amplification showed that adequate quantity of DNA for STR profile could be obtained from Chelex® extraction from 4 mL of urine from male volunteers, while Wizard®SV Genomic DNA Purification Kit did not give any amplifiable DNA. Also, cellular materials from 2-mL urine deposited onto FTA® cards gave amplifiable DNA but with inconsistency. The results obtained can be used to develop a guideline for performing DNA analysis as an integral procedure of urine testing for the anti-doping purpose, where cost per sample and storage space are factors of consideration.

Topic 06: Ethics & Legal

393. A MINI-PRIMER SET IN A MULTIPLEX PCR FASHION COVERING THE MTDNA CONTROL REGION FROM SKELETAL REMAINS SUBMERGED

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Skeletal often remains submerged in water yield DNA in a small quantity and low quality [1] in forensics studies. In such cases, although STRs are the gold standard for human DNA typing, alternative typing methods should be considered [2].Mitochondrial DNA (mtDNA) has proven to be an elegible source to the development of typing methods for human identification [3].The recommended approach to mtDNA analysis dwell in the PCR amplification of the entire control region (CR), a segment of mtDNA with 1122 base pairs (bp) [4]. However, when DNA is severely degraded, portions with such length may not be amplified [5]. To solve this, mini-primer sets multiplex reducing the amplicon sizes have been described sucessfully in amplifying the entire CR. Considering this approach, at least 5 overlaping amplicons are generated to cover the entire control region, overcoming the issues generated by the DNA degradation – as the fragmantation of the CR [6]. Therefore, this study aimed to develop a mini-primer set PCR multiplex, using 10 primers previously described in the literature, in order to amplify the entire CR of mtDNA from fragments of human skeletal remains sumerged in water up to 30, 60 and 90 days. The amplification of 3 samples submerged for 30 days have been analysed so far. All samples analysed showed sucessful amplification results. Furthermore, the findings suggest a great potential for use of the mini-primer set for the multiplex PCR assay developed.

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394. A SWISS COLLABORATIVE SIMULATED EXERCISE FOR DVI: LESSONS LEARNED USING THE FAMILIAS SOFTWARE

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Forensic genetic laboratories should be prepared as well as possible in their local region or country in case of a DVI (disaster victim identification) situation involving large-scale DNA profile comparisons. The 7 forensic genetics laboratories from Switzerland participated in a DNA profile matching exercise from a simulated plane crash on several houses, involving 70 victims. All profiles were generated using the program RStudio and the toolbox 'DNAtools' based on AmpFℓSTR® *NGM SElect*™ allelic proportions from Switzerland, as well as a co-ancestry coefficient of 1%. The post mortem database was comprised of 75 remain profiles. The reference database included 100 family references with diverse pedigrees (primary and secondary relatives), as well as direct references. The goal of the exercise was to correctly discover re-association and family matches. All participating laboratories used the DVI module of the Familias software, as well as one laboratory the Pedigree Searcher (CODIS 7), to compare the DNA profiles and assign likelihood ratios. The generated data was designed to challenge the participating laboratories, by including biological parents, children, siblings, secondary relatives, mutation events as well as non-paternity. In some cases the entire family was on the airplane and all were victims. Errors, pitfalls, statistical issues (prior- and posterior probabilities, likelihood ratios, accounting for the possibility of mutations, etc.) are discussed. In conclusion the DVI module of Familias (v3.2) offers many useful features and represents a valuable technological tool for rapidly and confidently contributing to identify victims of a mass disaster.

395. ALTERNATIVE METHODS FOR COLLECTION, ROOM TEMPERATURE STORAGE, AND PROCESSING OF DNA SAMPLES FROM HUMAN REMAINS: A NEW DVI APPROACH

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Mass disasters such as tsunamis and earthquakes often result in large numbers of casualties. If remains cannot be refrigerated or identified quickly, the body decomposes and the DNA in those tissues degrades and fragments making DNA typing more difficult. This project investigated the effectiveness of various simple in-field methods for collecting DNA from decomposing human remains. In addition, several room temperature DNA preservation, DNA purification, and amplification strategies were tested in order to facilitate faster and more direct identification processes for cases such as mass causalities in remote areas.

Skin and tissue samples were collected from three decomposing human cadavers over a two-week period in April at the Southeast Texas Applied Forensic Science Facility (STAFS) in Huntsville, Texas. Two methods were used to collect DNA from decomposing cadavers in the field: 1) skin/muscle biopsy, and 2) inserting a swab into a small incision in the thigh. Cotton, 4N6FLOQSwabs, and EasiCollect device with FTA Elute cards were tested. Biopsy punches were compressed onto FTA Elute cards prior to storage or stored in a tissue preservative solution that facilitates leaching of DNA into solution for quicker and more direct amplification. All samples were stored at room temperature for 1, 3, and 6 months.

DNA quantification and STR data will be presented to compare the success of each sampling, storage, and processing strategy. The results of this study demonstrate the potential of alternate and faster

sampling approaches for DNA human identification when environmental conditions are suboptimal.

396. AUTOSOMAL SINGLE NUCLEOTIDE POLYMORPHISMS-BASED INFERENCE OF RELATEDNESS FOR IDENTIFICATION OF MISSING CASUALTIES

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To identify the remains of service members missing in the Korean War, a population-based and DNA-focused identification system has been implemented since 2000 by the Ministry of National Defense in Korea. DNA profiles obtained from skeletal remains of Korean War victims are put into a database for random matching and kinship analysis to confirm the relationship between missing casualties and their alleged relatives. It has been reported that the problem of the possibility of erroneous match between unrelated people became significant especially with growth of the number of genotypes in A-STR database. To solve the problem related to high false positive rates, higher kinship index threshold is required. However, use of high kinship index threshold results in extremely high false negative rate. Therefore, the use of increased number of autosomal markers with minimal kinship index threshold is recommended. Highly effective massively parallel SNP genotyping platforms using microarray technology were developed and have been used in various human population studies. In this study, we adopted resequencing microarray platform to apply autosomal SNP markers to inference of relatedness between missing casualties and their alleged relatives. Reseuencing microarray showed reliable and robust results in sensitivity and degradation testing. Autosomal SNP markers analyzed using resequencing microarray platform showed remarkably higher likelihood ratios than 23 autosomal STR markers in kinship analysis of first to third degree of relatedness. Our results demonstrate that autosomal SNP markers hold great promise for identification of missing casualties.

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397. BEST PRACTICE RECOMMENDATIONS FOR THE ESTABLISHMENT OF A NATIONAL DNA IDENTIFICATION PROGRAM FOR MISSING PERSONS: A GLOBAL PERSPECTIVE

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I was awarded a 2015 Winston Churchill Travel Fellowship to visit international laboratories which have specialisation in the DNA identification of unidentified human remains (UHR), applied new technologies to DNA identification casework including the massively parallel sequencing (MPS) of mitochondrial DNA and forensic DNA phenotyping markers, and established successful DNA-led identification programs for missing persons (MP) casework or disaster victim identification. The goal of the Fellowship was to improve the DNA profiling outcomes for the 500+ cases of UHR in Australia, explore new technologies or DNA markers which could aid the identification effort in the absence of other investigative leads, and devise recommendations for the establishment of a DNA identification program focused on the 2000 long-term MP in Australia. Despite DNA being used worldwide to successfully identify large numbers of MP resulting from armed conflicts, human rights abuses and natural or man-made disasters, the cost, labour and success rate of using DNA for routinely identifying compromised UHR has historically been prohibitive for many countries, resulting in current backlogs of identification casework. The introduction of a nationally coordinated DNA testing program and adoption of DNA technological advancements, will facilitate the effective and efficient identification of a country's unknown and missing citizens thus bringing closure to potentially large numbers of missing person and criminal cold cases. I will translate key Fellowship findings into practical recommendations for the establishment of a national DNA identification program based on international best practice that will be applicable for any country considering implementing a DNA-led MP program.

398. DNA ANALYSIS FROM HUMAN SKELETAL REMAINS IN FORENSIC CASEWORK

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In cases involving charred remains, missing persons, and mass burials, highly degraded bone fragments are often the only obtainable physical evidence for human identification. To assess our laboratory's success with skeletal remains and provide a benchmark for the forensic community involved in identification of these remains, we retrospectively examined our ability to develop DNA profiles from the remains analyzed in our laboratory in the last 7 years. Between January 2009 and December 2016, 70 DNA extractions were completed on skeletal remains from routine casework. 92% of skeletal remains analyzed were samples submitted for body identifications by law enforcement and only 8% were samples submitted to answer family identity or historical questions. Overall, the ability to obtain a full or partial profile primarily reflects the difference in the average age and the condition of the samples in these two categories and thus, difference in the quantity and quality of the DNA. A femur was the most common sample (sometimes teeth, a rib or a skull). We describe here the approximate age and type of remains we have received, whether a full, partial, or no profile was obtained, as well as the condition of the samples (the environmental conditions from which the bone is recovered, burned remains or remains recovered from water). For body identification cases, when comparing genetic profiles, we matched 66 of the 70 skeletal remains analyzed with accompanying reference sample. The statistical analysis showed a high confidence of correct identification for all 66 victims (probability from 99.9% to 99.999999%).

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399. DNA PERSISTANCE IN SOFT TISSUE USING VODKA AS PRESERVATIVE AGENT

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In some crime scenes or following mass fatality incidents, human remains can be fragmented, burnt and/or decomposed, making victim identification by means of fingerprinting or odontology extremely difficult. In such situations DNA profiling is desirable; however, successful DNA analysis relies on the appropriate collection and preservation of biological material. Inefficient preservation methods can cause destruction of intact DNA to such an extent that data is not always available for victim identification. Biological samples have been successfully preserved using a number of physical and chemical treatments, adjusting temperature, ambient pH and salt concentrations. This study intended to assess the use of drinking alcohol as an alternative preservative solution for muscle tissue storage, comparing it with other preservative solutions. Pig muscle was incubated for up to 42 days at different temperatures (-20 °C, 25 °C, and 37 °C) with different preservative solutions: 95% ethanol, 37.5% ethanol, vodka (37.5%), and no preservative. Samples were collected weekly and analysis was based on DNA quantitation and amplification success of an in-house multiplex with amplicons between 70 bp and 384 bp. Samples incubated with 37.5% ethanol and vodka had high molecular weight DNA. All samples incubated with some kind of preservative solution generated complete profiles until the last collection point, while samples left untreated had drop-outs after 21 days of incubation at 25 °C and 37 °C.

400. DNA-ASSISTED IDENTIFICATION IN POST-CONFLICT ENVIRONMENTS

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The International Committee of the Red Cross is an impartial, neutral and independent organization created in 1863 to protect and assist the victims of armed conflict and other situations of violence and to promote the laws that protect victims of war; its mandate stems essentially from the Geneva Conventions.
Part of its work involves the search for people who have gone missing during conflict and other situations of violence. Acknowledging the increasingly important role of forensic science in the management of the dead and the identification of missing people the ICRC established its Forensic Services in 2003. The aim was to provide forensic assistance where required to locate, recover,

document, manage and ultimately identify individuals that have gone missing through conflict and other situations of violence; the remit has expanded in recent years in the form of humanitarian forensic action, applied also in situations of natural disasters and migration.
The ICRC's Forensic Services is active in approximately 40 contexts, with Forensic Advisors *in situ* to coordinate support. In many of the contexts the use of DNA to assist with the identifications of missing persons is either planned or ongoing. The ICRC's support for DNA-assisted identifications includes providing advice and training, assisting the authorities with Biological Reference Sample collection, and material assistance. An overview of activities with some illustrative examples will be presented.

401. DO WE REALLY NEED TO CRUSH? AN ALTERNATE DNA EXTRACTION APPROACH FOR BONE SAMPLES

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Bone samples are often encountered in missing persons' cases and mass disasters for human identification purposes. Downstream STR typing success is dependent on the quantity and quality of DNA retrieved from these samples. Traditional bone DNA extraction methods rely on cutting and crushing bone into a fine powder and a long demineralization step coupled with an organic or silica-based DNA purification method.
The TBone Ex kit (DNA Chip Research Inc.) in combination with the PrepFiler® BTA Forensic DNA kit (ThermoFisher Scientific) is an alternate approach which avoids powdering bone samples prior to purification. We compared the effectiveness of this method to a complete demineralization method[1] and the standard PrepFiler BTA protocol using powdered bone (with/without automation).
Bone samples (N=6) from human cadavers exposed to various insults (fire, decomposition, sun exposed, burial, embalming) were prepared, extracted and STR-typed in triplicate. Although DNA yields were higher using the complete demineralization protocol, STR success rates and overall profile quality were comparable across the manual methods tested.
The TBone Ex system is compatible with Prepfiler BTA chemistry and is an alternative method to conventional complete demineralization methods which are more laborious and time consuming (~30hrs versus 16-19hrs), require powdering of bone tissue, pose a higher risk of contamination, and consume the entire sample. The main advantage of the TBone system is the provision for a second DNA extraction from the same sample. These extracts may be used to perform additional analyses, or combined and concentrated to increase the amount of input DNA for improved results.

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402. EVALUATION OF DECALCIFICATION FOR RECOVERY OF DNA FROM BONE

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Decalcification has been used as a step in the DNA extraction process to release DNA embedded in the dense crystalline matrix. However, the decalcification process adds more handling and pipetting steps

thereby increasing the extraction time, cost and also the potential for contamination.
Decalcification using 0.5 M EDTA was carried out on fresh bone samples and extracted using the phenol-chloroform-isoamyl alcohol extraction method. The same fresh bone samples were also extracted without decalcification. The final volume was standardized at 100 µL for comparison. After the extraction, quantification was carried out on all extracted samples using GoTaq® qPCR Master Mix quantification method with non-fluorescent forward and reverse primers to give an amplicon of 70 bp.
Even though there are studies showing DNA recovery is better with decalcification (1-2), high DNA yields were extracted without decalcification. This study showed that decalcification is not a necessary step in the extraction process when using good quality samples and the DNA yield was higher when the decalcification process was eliminated, presumably because free DNA is not being washed away. In addition, good quality electropherograms were produced from the extracted DNA samples without decalcification.

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403. FORENSIC PERFORMANCE OF A VERY LARGE HUMAN IDENTITY SNP PANEL

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We designed a very large SNP panel for MPS applications to missing persons identification, with the aim of abetting genetic data recovery from small genomic targets suitable for degraded samples, and to permit powerful kinship statistics for matching to distant family references. 1457 loci are targeted, including 1411 tri-allelic or tetra-allelic SNPs and 46 autosomal microhaplotype loci, with a target fragment size of ~75 bp or less. A multiplex target enrichment MPS assay was developed based on Qiagen QiaSeq chemistry. The library preparation starts with ligation of random unique barcodes to each fragment of target genomic DNA, which, after sequencing, permits the differentiation of authentic variants from variants that result from PCR error. Enrichment is achieved with the use of extension primers specific to binding sites near each SNP target, and PCR amplification of genomic fragments using the target-specific extension primer and a universal primer whose binding site was incorporated into the ligated barcode/adapter. For almost every SNP, two target-specific primers were designed, each chosen to lie within ~75 bp of the SNP (and in many instances much closer). These primers do not together amplify the SNP locus as a pair, but instead provide redundant targeting of the desired SNP. The final multiplex contains 2832 target-specific primers. Results will be presented on the performance of the assay on various sample types, including degraded bone samples, using both the Illumina MiSeq and Qiagen GeneReader instruments.

404. GENETIC ANALYSIS OF OLD SKELETAL REMAINS FROM

KOREAN WAR VICTIMS USING POWERPLEX® FUSION 6C AND MINISTR SYSTEM FOR HUMAN IDENTIFICATION

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To analyze and identify the population of the Korean War victims by studying degraded old bones based genetic analysis, we examined 108 unrelated individual femurs except two low quality bones from total investigated 110 samples. In order to obtain significantly improved genetic profiles, amplification was performed using recently developed commercial PowerPlex® Fusion 6C system and miniSTR system.
We have modified PCR condition to gain enhanced DNA profiling at a success rate. When each value of two different systems came out respectively as 92.35% and 91.05% only, it created 4.40% increased value which rated at 95.45% by using these two systems simultaneously. Especially, 100% showed at D1S1656, D2S441 and D12S391, which overlapped with miniSTR system. Total of 24 autosomal STRs had gene diversity values from 0.7751 to 0.9934; SE33, Penta E, and D2S1338 which were the best in that order. The most frequent value was 0.5170 at TPOX(8) in the frequencies ranging from 6 to 37 allele. Polymorphism information content, power of discrimination and exclusion, typical paternity index and heterozygote were the highest value at SE33, but TPOX appeared the lowest in all statistics. However the value of matching probability and homozygote showed the opposite result. Since above data anticipate that these outcomes confirm the similar patterns compared to previous reported Korean population papers, it verifies the population based genetic analysis using advanced these two systems is very helpful to increase an efficiency as well as a higher accuracy on identification of victims and also to check of identical samples.

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405. MASSIVE IDENTIFICATION OF VICTIMS OF TOTALITARIAN SYSTEMS AND HOLOCAUST IN POLAND

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We would like to present results of 5 years of research conducted within the project of the Polish Genetic Database of Totalitarianisms Victims. The project was created in 2012 for the purpose of collecting of the reference material from living relatives of the victims. As part of the project, over 800 human remains discovered in nearly a dozen sites all over Poland are being analyzed. To this day,

more than 70 victims were identified and for about 400 of analyzed remains, we already completed the genetic study. Initial research was focused on identifying victims of the communist system in Poland, but it soon became clear, that the project can also be used for the victims of Nazis. Therefore currently within the project we are studying remains of victims of communist crimes - found in Warsaw, communist and German Nazi crimes – in Białystok and victims of Holocaust, whose remains were found in both Sobibor Concentration Camp. In our study we use a set of autosomal STR markers, STRs located on sex chromosomes, we also analyze sequences of mtDNA's. In addition, we implemented the next generation sequencing technology into our project. In human remains analysis, apart from the personal identification itself, haplotyping, based on the analysis of haploid markers - Y-chromosomal STRs and mtDNA sequence - is becoming more and more essential. It sometimes occurs that at one site we find victims of different regimes. Haplotyping of those victims buried in mass graves helps to determine the origin of the crime.

406. MISSING PERSON AND DVI WITH CLOUD BASED SOFTWARE

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The identification of missing person or unknown remains and the handling of disaster victim identification (DVI) can be a tremendous challenge. During this type of cases a set of remains is being compared to many samples, which cause a higher level of complexity and challenge of kinship analysis. Also these types of analysis are not a typical daily work for a forensic laboratory.
To enable a fast analysis of human remains or DVI cases we developed the cloud based software Genolab (www.genolab.eu). The combination of specially designed biostatistic algorithms and method with the advantages of cloud technology provides the capability to analyze also cases with several hundred or thousands of DNA profiles.
We show as an example the analysis of remains of a medieval cemetery. The found skeletons date from about 990 to 1260 AD and were in a bad condition. The DNA analysis of the remains was carried out by various Saxon universities. On the basis of this data a kinship analysis with Genolab was done to answer different questions: For example, whether the found children skeletons may be related with found adult remains.
These examples will demonstrate how cloud computing, combined with new biostatistical 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.

407. RECOVERY OF EXOMES AND MITOCHONDRIAL GENOMES FROM DENTAL CALCULUS

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Calcified dental plaque (dental calculus) can be removed from dentition without damaging the underlying teeth or surrounding bone and it has been shown to be an excellent reservoir of microbial and dietary biomolecules, microfossils, and host DNA. Here, we use capture and next-generation sequencing (NGS) to examine the preservation of mitochondrial and autosomal DNA in dental calculus

from 42 skeletons recovered in Tanzania and Puerto Rico dating 4 - 51 years and 700 - 2,500 years before present, respectively. Specifically, DNA was extracted and built into double-stranded shotgun libraries before being amplified and undergoing in-solution hybridization capture enrichment and sequencing (Illumina MiSeq, 2x150pe). Capture and sequencing of mitogenomes from 42 calculus samples (as well as 7 dentine samples from a subset of these individuals) showed that at least 99% of the genome was recovered across most samples with ranges of 11x-105x for calculus and 158x -315x for dentine. In addition, after exome capture (using IDT xGen Lockdown Probes and Reagents) along with NGS (Illumina HiSeq Rapid 2x100), we obtained genetic data from 2 calculus and 1 dentine sample from 2 individuals. Our preliminary results show that a single round of sequencing produced 225,000-570,000 callable sites within the exome, covering 13-61% of all capture targets. Our results suggest that dental calculus can be an important source for DNA recovery in forensic studies.

408. WHAT WE CAN LEARN FROM ANCIENT BONES – Y-STR ANALYSIS OF HUMAN SKELETAL REMAINS FROM THE AMERICAN CIVIL WAR, WORLD WAR II, SEVEN YEARS' WAR, AND THE AMERICAN OLD WEST

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Bones are a valuable source of DNA from decomposed human remains for forensic, anthropological, and archaeological investigations. Y-STR analysis represents an important tool to complement autosomal DNA analysis and to help determine kinship relationships among bone samples and living descendants. In this study, we describe the preparation, DNA extraction and Y-STR typing of a variety of human skeletal remains, including samples from the American Civil War (1861-1865), World War II (1939-1945), Seven Years' War (1756-1763), and the American Old West (Deadwood, late 19th century). Bone samples were first surface-sanded, sectioned, and washed. Dried bone cuttings were ground into powder and DNA was extracted using a variety of organic and silica-based methods. Y-STR typing was performed using the Yfiler™ and Yfiler™ Plus kits on the 3500xl Genetic Analyzer. The Yfiler Plus kit includes the 17 markers from the Yfiler kit plus 10 additional highly polymorphic Y-STR markers (DYS576, DYS627, DYS460, DYS518, DYS570, DYS449, DYS481, DYF387S1a/b and DYS533). Data were analyzed using GeneMapper™ ID-X Software v1.4. Regardless of the DNA extraction method used and the age of the specimen, samples analyzed with the Yfiler Plus kit yielded a greater number of alleles as compared to samples typed with the Yfiler kit, indicating a better ability to analyze degraded DNA and overcome inhibitors. A complete analysis of the DNA extraction methods as well as the Y-STR typing results will be presented, demonstrating the value of the Yfiler Plus kit in these types of investigations.
For Research, Forensic, or Paternity Use only.

409. BODY FLUID STAIN AS A FORENSIC OBJECT: IN THE SHADOW OF ENORMOUS ACHIEVEMENTS IN DNA ANALYSIS OTHER ESSENTIAL ASPECTS OF THE EXAMINATION CAN

BE ECLIPSED

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The key to a successful crime investigation is effective work with forensic information. However, essential complication of forensic technologies, requiring the involvement of a number of professionals specializing in narrow research, result in excessive differentiation of modern forensic knowledge. Further, because of enormous achievements in DNA analysis other ways can be eclipsed in practice to study the forensic body fluid stain, important for the assessment of the forensic significance of the stain for the case in hand. So, according home statistics, BPA is appointed incomparably less often than DNA analysis and serological examination of bloodstains. This leads to the fact that identification is performed, but the mechanism of the trace formation is not taken into account. Under these conditions there may be a disadvantage due to the loss of the vision of the integrated information picture which is necessary for the successful crime investigation. The integrating view is necessary both for the forensic examination and the crime investigation levels. The lack of such vision can result in the failure to use all the potential of the available expert arsenal to solve the tasks of the particular investigation; inappropriate appointment of the succession of forensic examinations without taking into account the destructive/non-destructive nature of the methods; inefficient use of the stain's material; poor data interpretation, etc. The total algorithm of the forensic examination should take into consideration the entire spectrum of the informative fields of the object under study which are relevant to the particular case.

410. CHALLENGES OF COMMUNICATING FORENSIC GENETICS TO MULTIPLE PUBLICS

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The use of DNA is gaining a prominent role in criminal investigations. Public perceptions are increasingly shaped by imaginaries that present DNA as infallible evidence to solve crime. This context is prone to misinterpretation and to raising unrealistic expectations around the potentialities of DNA technologies. In this paper, we explore the views of forensic experts about the challenges of communicating the potentialities and limitations of forensic genetics to multiple publics. Our data draws on semi-structured interviews with 20 forensic experts professionally working and accompanying the uses of DNA in the criminal justice systems of different European countries.

Recognizing the need for social accountability, forensic experts actively communicate with two main audiences: the *specialist public* of the criminal justice system, who professionally engage with forensic science; and the *distant publics*, a blurry field composed of media, civil society stakeholders and ordinary citizens.

Our data shows that the recognition of the complexity of publics and the challenges to address them give rise to three interrelated strategies of communicating forensic genetics. First, to convey in an accessible manner the specificities of knowledge production in forensic genetics, creating a common ground for an informed discussion. Second, to address diversified views and expectations of multiple publics, characterized by different levels of information. Third, to (re)build public trust in experts and organisations involved in forensic genetics, in a way that expands the conventional forensic practitioners' role towards public educators and reinforces the field's contribution to responsible governance of information.

411. HOW DOES FORENSIC SCIENCE FIT INTO KOREAN SOCIETY?

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Forensic science involves not only using human biological materials but also storing, managing and using information obtained from human derivatives and information obtained from them. This necessitates consideration of the ethical, legal and social implications of studies of humans and use of results from these studies, as in other medical and life-science studies.
The data were collected as part of the Korean Academic Multimode Open Survey (KAMOS). Of 2,000 randomly selected panel members, 1008 respondents participated in this survey. Eighty-nine-point-two percent of the respondents approved the implementation of the criminal database for the purpose of arresting offenders and preventing recidivism. In addition, 70.8% of the respondents agreed with the expansion of the crime categories entered in the criminal database. Generally our results suggest that the current forensic DNA database is considered highly trustworthy. Many of the respondents (93.4%) favored genetic testing and data storage to determine the identity and the cause of death for a person who has died of unnatural causes. However, 35.1% of respondents expressed concern that the development of forensic science techniques limits personal information and physical freedom.
The aims of this study were to draw a broader picture of the public opinion on the DNA database and indicated what social impact forensic science can make, and what possibly can be done to make forensic science fit in with society. Our data might provide an additional perspective, how society may view such an impact, for forensic experts.

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412. ON THE STANDARD OF THE GENETIC IDENTITY DETERMINATION ON THE BASIS OF THE CONSENSUAL LEVEL OF RELIABILITY OF DNA IDENTIFICATION

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Since neither forensic scientists nor judges have absolute criteria for the evaluation of the probability values, there is a room for the subjectivity of the assessment of the DNA analysis results. Despite each case is unique in its own way, fundamentally it is possible to define a scientifically ground threshold of identity and accept it as a standard as suggested by Perepechina (2002). The standard should be conservative in order to provide a reliable solution concerning identity regardless of the circumstances of the case. In the proposed model, with later refinements (Perepechina, 2014), the DNA identification standard is to be developed on the basis of the predetermined high consensual level of reliability, justified by the scientific community, which exactly meets the requirements of the national judicial

system - this is the key point of the concept. As a technology of the choice of the standard, an analysis of a series of the equivalent probabilities is proposed which provide the assessing the risk of the identification error from different sides and, in some way, “visualize” it to those who make a choice of the criterion. In the current report we present an updated concept and discuss the aspects that have not been considered earlier. The outlined approach may be applicable not only to DNA analysis, but also to other areas of forensic identification.

413. YOUNG PEOPLE'S PERSPECTIVE ON THE ITALIAN FORENSIC DNA DATABASE: RESULTS OF A PILOT STUDY ON UNIVERSITY STUDENTS

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In Italy, little is known about the public awareness also of National Forensic DNA database and its acceptance among citizens. In order to provide an empirically-grounded assessment of individual perceptions and collective attitudes to the risks and benefits of forensic DNA databases, a questionnaire was carried out to a group of students of Padua University. The questionnaire comprised questions covering the level of knowledge about the Italian forensic DNA database, the perception of the benefits and risks of this institution, the attitudes towards to have their own DNA profile included in this database and the views informing such an opinion. The large majority of students who were asked to participate in the survey answered to the questionnaire and the number of participants (N=980) could be considered representative of the target population. The 35% of the participants expressed their choice to accept having their profile insertion in the National Forensic DNA Database, even if they are not aware of the benefits and risks of such action. The percentage of the responders accepting of having the genetic profile included is higher among those who answered that forensic DNA databases are a powerful tool for improving the efficiency of the criminal justice system. This study may indicate the knowledge of citizens about the field of forensic DNA databases and the general attitude of Italian people with higher levels of education to have the genetic profile included in the National Forensic DNA Database.

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414. CHALLENGES AND CHANCES OF FORENSIC DNA ANALYSIS AND DNA DATABASE IN KOREA

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Forensic DNA analysis in Korea went back to the year of 1991, when the first forensic DNA laboratory was opened at National Forensic Service. Forensic DNA technologies rapidly expanded during 2000s by development of new autosomal STR multiplex kits, Y and X chromosome STR kits, and mitochondrial DNA sequence analysis, which became the most powerful tool in forensic disciplines.
Last year NFS analyzed over 200,000 samples from crime scene and arrestees. Since 26th of June, 2010, NFS and SPO have operated National Forensic DNA Database (NFDD) together, which changed the paradigm of crime investigation in Korea. It was determined to expand DNA database core loci from 13 to 20 within this year to reduce the probability of random match. At 2015, the statute of limitation on homicide was removed, so hundreds of unsolved murder cases could be re-investigative.
Future technologies such as prediction of age and body fluids identification by DNA methylation analysis, prediction of bio-geographical ancestry and visible characteristics, multiple parallel sequencing and rapid DNA technologies opened new era in forensic DNA, however several legal and ethical issues were aroused.

415. DNA DATABASE OPERATION AND HIT CASES IN THE KOREAN PROSECUTION

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In Korea, DNA database(DNA-DB) has been operated since July 2010, in accordance with the legislation of "DNA Act" in December 2009.
DNA-DB is managed separately by the prosecution and the police according to the kind of data to be constructed. The prosecution manages offender's DNA profile and personal information with consideration of human rights and policy, and the police manages DNA profiles of evidences and suspects. Two separated databases are connected electrically, and prompt search followed by notice goes on.
Here, we introduce STR marker expansion in DNA-DB of the Korean Prosecution. Thirteen CODIS core loci were used since the database has been established. Therefore, the more database becomes bigger the more necessity is reconsidered. We've prepared for using 20 CODIS core loci with the police and National Forensic Service(NFS). We validated commercial kits of domestic as well as foreign which can analyze 20 expanded CODIS core loci. Also we've applied original frequency by analyzing frequency of each marker with 2,000 Korean populations.
DNA-DB of the Korean prosecution service manages over 120,000 offenders during 7 years, almost 20,000 offenders are annually added. It is very useful and helpful to resume investigation of cold cases also security of evidences with informing the matches that searched between crime scene and offender's DNA-DB to investigators. Our database is operated well with consideration of critical issues such as STR marker expansion the major issue of world DNA database progress trends, furthermore, we'll further its practical usage of international investigation by building international corporation network.

416. DNA PROFILING DATABASE: FUNGI ATTACK

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The Central Institute of Forensic Science (CIFS) has established a DNA database since 2004. Currently, CIFS plans to expand size and capacities of the database in order to monitor repeated crimes and support law enforcement agencies in justice system. In the period of 2015-2016, 18,000 prisoners were collected buccal cell on Whatman® Indicating FTA® card. Unfortunately, approximate 440 samples were appeared fungi-growing on FTA card (2.5%). Preliminary, 195 samples were selected as a pilot project to perform DNA typing using GlobalFiler™ Express PCR Amplification Kit with validated protocol. Full profile was detected from 124 samples with first punch of 1.2 mm diameter FTA card and other 42 samples were passed with second punch. In the other hands, 20 samples were DNA extracted with whole piece of FTA card and obtained full profiles. In summary, 186 full profiles were recovered as 95% success rate, 4 partial profiles and 5 samples with no profile. However, the sample without appearing of fungi is more suitable for DNA genotyping. Thailand is located in the tropical area, presumably, heat and humidity may be suitable conditions for promoting fungi growth. Other factors suspend to be cause of fungi growth such as collecting kit, sample collection method, preserve condition, shipping method and delivery time frame etc. In order to prevent this obstacle CIFS Laboratory and the collector should have collaboration to find out an appropriate way to solve the problem. Moreover, FTA collecting kit failure needs to be further investigated.

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417. EFFECTIVENESS OF CIFS DNA DATABASE IN THAILAND

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In 2004, the Central Institute of Forensic Science (CIFS) established DNA database. The DNA database originally utilized as intelligence to support law enforcement agencies to solve organized crimes in Deep South of Thailand. That area was covered three provinces border between Thailand and Malaysia. There are briefly 1,600 hits found from various incidents of that area. Recently, additional offences were kept in the CIFS DNA database such as homicide, murder, sexual assault, burglary and drug smuggling. Until now March 2017, CIFS DNA database held approximately 145,000 DNA profile records included 130,000 records of individuals (potential suspects and prisoners) and 14,000 records from crime stain samples. Autosomal STR markers were mainly analyzed and collected in the DNA database. Y-STR markers also were analyzed and uploaded especially crime stains from sexual assault cases and their potential suspects. Other 200 hits were reported on the additional offences. Almost 2,000 hits were found and reported to law enforcement agencies. CIFS plans to expand the capabilities in DNA investigation such as collecting new registered prisoners and all prisoners in Thailand in order to increase hit rate and monitor repeated crimes. CIFS DNA database also prompts to work on DNA searching and data exchange for national and international law enforcement agencies. An ultimate goal of DNA database is to help to solve transnational crimes such as human trafficking, migrant smuggling and illegal labors.

418. INCREASING THE EVIDENTIAL WEIGHT IN RELATIONSHIP TESTING BY TAKING STR DNA SEQUENCE POLYMORPHISMS INTO ACCOUNT

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The recent year's development of massively parallel sequencing (MPS) instruments and assays have now made it a compatible complement to the established capillary electrophoresis (CE) analysis for different forensic genetic applications. It is well known that short tandem repeat (STR) alleles of the same fragment size could have different DNA sequences. Thus, there will be an expected increase in the population genetic diversity for the present set of forensic STRs when performing the analysis with MPS technologies. In order to study the additional value of this increase of information for relationship testing, we set up an allele frequency database for the Swedish population for the markers included in the ForenSeq DNA Signature Prep Kit (Illumina). Three hundred individuals with Swedish origin were analyzed and allele frequency distributions for 58 STRs were established. In addition, simulations were performed in order to study the impact of this observed increased number of alleles for the expected likelihood ratios (LRs) for different kinship case scenarios. To conclude, this study provide guidelines for which type of case scenarios that the additional DNA sequence information will be useful, and the establishment of the allele frequency database will enable biostatistical calculations to be performed in casework.

419. MOVING TOWARDS A SHARED FORENSIC STANDARD IN ITALY

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One of the main gool of the European Union is to promote common actions between the Member States in the field of police and judicial cooperation in criminal matters.
It will entail a cooperation of the EU Member States to ensure the even-handed, consistent and efficient administration of justice and the security of citizens in the area of forensic science. In 2011 the EU Council [1] formed the ambition to create a European Forensic Science Area by 2020.
These indications have been collected from Italy by joining the Prűm Decision with the 30 June 2009 legislation n. 85 concerning the analysis of DNA, fingerprints and vehicle registration rules. The implementation of DNA legislation in Italy started seven years later, with the DPR 7 April 2016, n. 87 that indicates the necessity of the ISO/IEC 17025 standard for the laboratories that aspire to send DNA profiles to the Italian DNA Database. Thus, it is obvious that the accreditation of forensic DNA laboratories is a corner stone of the Vision of European Forensic Science 2020. In the same time the Italian forensic police laboratories have now reached the ISO/IEC 17025 accreditation for DNA implementing their quality system with fingerprints, drugs, gunshot residues. By contrast very few university laboratories have followed this quality evolution, which certainly requires economic investments.
Here, the effect in the Italian legal framework will be discussed, regarding the possibility to ensure

effective adversarial in criminal proceedings.

1. Council 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.

420. PHYLOTREE BUILD 18: UPDATE OF THE HUMAN MTDNA PHYLOGENY

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Considering the evolutionary aspects of human mitogenome variation can aid forensic mtDNA analysis in several ways: phylogenetic analysis can point to potential sequencing errors; homoplasy levels allow the estimation of site-specific mutation rates; and haplogroup inference reveals a person's matrilineal biogeographic ancestry. For these applications, an accurate and comprehensive phylogenetic tree of global human mtDNA variation is essential. In 2008, the first version of PhyloTree was released at http://www.phylotree.org [1]. This tree has subsequently been regularly updated, bringing its overall resolution from a total of 1267 haplogroup nodes in Build 1 to a total of 5437 nodes in Build 17 (18 Feb 2016) [2]. 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 [3], HaploGrep [4] and Phy-Mer [5], among others [6]. This poster introduces the newest version of PhyloTree, Build 18.

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421. POLICE OFFICERS DNA ON CRIME SCENE SAMPLES - INDIRECT TRANSFER AS A SOURCE OF CONTAMINATION AND ITS DATABASE-ASSISTED DETECTION IN AUSTRIA

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The capability of nowadays used forensic DNA analysis techniques to generate DNA profiles out of

evidence samples that contain only a low amount of biological material has also enhanced the chance to detect unwanted contamination incidents [1]. The identification of such contamination events to reduce false positive results and to avoid misguiding investigations still represents a challenge for forensic laboratories [2]. Especially the complexity and different possibilities of DNA transfer make the identification of contamination incidents quite difficult as biological material can not only be transferred by means of direct but also by means of indirect transfer. Direct transfer or primary transfer not only includes direct contact such as touching but also activities (e.g. speaking, sneezing, coughing) in the immediate vicinity of an object. Indirect transfer, also called secondary, tertiary etc. transfer, means the transfer of biological material via multiple steps (e.g. from an individual by means of direct transfer to an item and than from this item to another item) [2, 3, 4].
With improved methods to detect contamination incidents it becomes apparent that indirect transfer of biological material during crime scene investigation is a serious issue [5, 6].
By presenting different cases of indirect transfer of biological material from police officers during crime scene investigation we would like to highlight the benefits of database-assisted detection methods. Without the help of databases containing reference profiles of police officers for automatic elimination, the majority of contamination incidents caused by indirect transfer would have remained undetected.

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422. SEARCH, ALIGN AND HAPLOGROUP – IMPROVED FORENSIC MTDNA ANALYSIS VIA EMPOP

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The analysis of human mitochondrial DNA (mtDNA) has proven to be extremely useful in several forensic and population genetic applications and also gains importance in disease studies. Conventionally, the obtained nucleotide sequences are aligned relative to the corrected version of the first sequenced human mitogenome and only the differences at a given position are noted and determine the haplotype.
However, a major limitation of this very common approach is the fact that difference-coded haplotypes may be ambiguous because often more than one single alignment is feasible. Consequently, database searches for forensic frequency estimates that are performed with difference-coded haplotypes are susceptible for biased results, typically the underestimation of the frequency of an mtDNA haplotype. The use of string-based search algorithms that convert query and database profiles into position-free nucleotide strings constitute an important alternative to avoid such database search bias.
The new search software presented here provides this functionality with the additional feature that sequence strings can be translated into phylogenetically aligned difference-coded haplotypes, why the

tool can also be used to harmonize nomenclature between different laboratories. In addition, the presented software allows for haplogroup estimation based on Phylotree, representing a comprehensive phylogenetic tree of worldwide human mtDNA variation that is regularly updated.
The new concept extends a previously presented string search algorithm that was implemented in EMPOP in 2010 and represents a comprehensive and user-friendly tool for human mtDNA analysis based on Phylotree Build 17.

423. THE BRAZILIAN DNA DATABASE: A VIEW AFTER 04 YEARS

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The Brazilian DNA database network was established in 2013 and is now composed by 19 DNA labs (18 state and 1 federal DNA lab). All the technical procedures are established by a Federal Executive Committee, composed of 11 representatives from the federal and state governments as well as human rights. More than 200 investigations have been aided since the DNA database launch, one of which involved a wrongly accused suspect. Most cases are either sexual violence or property crimes.
However, the numbers of convicted fellons profiles included in the database is still low, especially in a country known by its huge rates of violence and incarceration. One of the main reasons for this extreme delay is the resistance from some criminal prosecutors and law enforcement professionals who refuse to allow DNA collection from convicted offenders, without their consent, claiming this procedure is unconstitutional.
Recently a new fact has emerged that can help change and leverage the law and consequently the Database: the Supreme Court is analyzing a Petition where an State Public Defender questions the constitutionality of the DNA law. As the issue is very new to the Court a group of forensic scientists has been called to assist with technical and scientific fundamentals as *amicus curiae* (Latin term meaning "friend of the court").
In a country with more than 50% of prisoners awaiting judgment the correct use and expansion of the DNA Database can be extremely positive to avoid testimony-only based arrests and absence of physical evidence on trials.

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424. THE POWER OF FORENSIC DNA DATA BASES IN SOLVING CRIME CASES

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Forensic DNA databases constitute an important investigative resource in contemporary criminal justice systems. The centralised and computerised storage of DNA profiles in a database enables the systematic comparison and automated matching of crime scene samples and individual profiles. Many countries operate forensic DNA-databases to identify owners of crime related stains. Using DNA to trace people who are suspected of committing a crime has been a major advance in policing. When DNA profiling is used wisely it can help to convict people who have committed serious crimes or exonerate people who are innocent. A DNA database is a computer database containing records of DNA profiles. Usually there are two different sources of these DNA profiles: crime scene DNA samples and individuals' DNA samples. The use of DNA databases in criminal investigations requires an individual's identity to be revealed only if there is a match between their DNA profile and a crime scene DNA profile.
We present two homicide cases where killers where identified by comparing the unknown STR's profile found on ropes, which were used to tie the victims, with Macedonian forensic DNA database stored in Forensic department of MIA. Statistical analyses were performed using DNA View software

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Topic 07: Predictive Markers

425. A SHORT UNIX SHELL SCRIPT FOR VCFTOOLS COMMANDS ITERATION TO OBTAIN THE GENETIC CHARACTERISTICS OF VARIANTS FOR FORENSIC PURPOSE

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The release of 1000 Genomes Project phase 3 variants database has provided a great variety of genome-wide variants spanning major populations worldwide, which offered a convenient path to the study of human genetic structure. VCFtools, a suite of functions dedicated to loci details summarizing, calculating, filtering, and genotype outputting from available vcf (variant call format) files released from 1000 Genomes Project, can act as a powerful utility for genetic markers hunting and evaluating in the context of forensic purpose, e.g., biogeographical ancestry (BGA) inferring, forensic DNA phenotyping (FDP), as well as forensic DNA typing panel constructing. While it is a labor and time consuming, as well as error-prone job involving repeating of multiple VCFtools commands by altering some arguments, e.g., populations, chromosomes, and positions, etc., during the process of loci summarizing and filtering step by step. Hence, we introduced a short UNIX-based bash shell script which contains an iteration structure to call VCFtools commands repeatedly for summarizing of allele frequencies, chromosome positions, linkage disequilibrium statistics, heterozygosity, etc. in different loci of different populations, filtering under certain criteria such as minor allele frequencies, allele counts, variant types (SNPs/InDels), and chromosome positions, as well as individuals' genotype outputting automatically. By setting up loop condition and arguments list, VCFtools commands with different arguments can be executed consecutively. Consequently, this process can be realized on an unattended computer with high efficiency and precision in the study of forensic genetics.

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426. ADOPTION OF THE DECISION CONCERNING GENETIC IDENTITY IN THE FRAMEWORK THE DECISION THEORY : VERIFICATION OF THE MODEL PARAMETERS BY EMPIRICAL DATA

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Along with the assessment of the objective statistical value of reliability of DNA identification discussed elsewhere, the problem of choosing the criterion for establishing the genetic identity also includes the aspect related to the subjective factor - the adoption of the decision regarding the identity threshold. The decision-making point is unavoidable at any degree of reliability of the DNA identification. As suggested by Goubko and Perepechina (2001), decision theory provides solid ground to recommendations on forensic DNA identification use in legal studies. The developed mathematical model based on DNA information, describing forensic aspects of decision theory application to court decisions, employs hypothesis of the rational behavior to reveal factors influencing decision-making, and to study border probability values, which can be assessed as sufficient for the judgment. The key element of the model is the empiric adoption the level of type I vs type II judgment errors by the society. The later research (2015) showed the adequacy of the model parameters to the realistic values of contemporary DNA analysis. In the current report we concentrate on the stage of the obtaining and discussion of the empirical data necessary to study sociological aspects of the problem and draw valid conclusions concerning the method. The opinion poll is in progress and allows to make noteworthy observations.

427. CHALLENGES IN LT-DNA MIXTURES INTERPRETATION: A PROOF-OF-CONCEPT MULTI-KITS, MULTI-INSTRUMENT AND MULTI-SOFTWARE COMPARISON WHEN APPROACHING HIGHLY CHALLENGING SAMPLES

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Recently the technical improvements in forensic genetics allowed the analysis of few DNA picograms collected from trace evidences. Since obtained profiles are often DNA mixture, probabilistic software are essential to interpret such challenging samples. Nevertheless, in this study we evaluated how probabilistic software may affect the final results in terms of interpretation performances on Low-Template (LT) DNA mixtures obtained using several commercial typing kits and different generation of CE instruments. Five biostatistical software, such as Lab Retriever[1] and LRmix Studio (semi-continuous algorithms)[2] – and DNA•VIEW®[3], EuroForMix[4] and STRmixTM[5] (fully-continuous algorithms) were employed to calculate likelihood ratio (LR) values for a series of self-prepared DNA mixtures containing 2, 3, 4 and 5 known contributors, in different proportions and dilutions. DNA mixtures were amplified with seven DNA amplification kits to evaluate whether the kit selection represents a bias factor. Same evaluations were performed using different instruments such as 3500 and 3130 Genetic Analyzers. Multi-software approach helped us to highlight trends in LR results. Up to now, fully-continuous computations provided different results in terms of LR magnitude's degrees values when compared to semi-continuous ones. Both typing kits and instruments, used critically, affected the results regarding minor contributors in highly unbalanced mixtures and LT-DNA. As a proof-of-concept study, this work aimed to emphasize the joined use of new CE and kits with fully-continuous models, especially in case of LT-DNA mixture interpretation. Previous generation kits and instruments combined with semi-continuous software might provide inconclusive and/or inappropriate results. Anyway, every protocol has to be deeply validated.

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428. DNAMATCH2: AN OPEN SOURCE SOFTWARE FOR CARRYING OUT LARGE SCALE CONTAMINATION SEARCHES USING QUALITATIVE AND QUANTITATIVE MODELS

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A very fast, but efficient, contamination search algorithm has been implemented as the open source R-package *dnamatch2*. Both "stain-against-stain" and "reference-against-stain" comparisons are carried out, where the stains can be partial mixture profiles with allele drop-out. First, matching allele counts are very rapidly carried out on all comparisons to provide a list of candidate matches and then likelihood ratio statistics are applied for the remaining candidates - reducing the number of false positives. The likelihood ratio statistics are first based on a qualitative model (similar to LRmix Studio) and then a quantitative model (*EuroForMix*). These are employed sequentially since inference of the qualitative model acts as a primary filter and is much faster than the quantitative model.
The input of the algorithm provides great flexibility to the user: The name structure of the files, required thresholds in order to a potential match, peak height threshold, stutter-threshold and expected amount of drop-in. The motivation for the software is contamination search used for quality control but it can also be used for large database searches of contributors to complex mixtures. We provide an example where the software has been important in order to discover a contamination.

430. EVALUATING THE PERFORMANCE OF FORENSIC GENETICS STATISTICS IN BRAZILIAN POPULATION

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DNA typing is a powerful tool in criminal cases. After comparing genetic profiles, statistical methods are applied to calculate the likelihood ratios (LR) considering the probability of DNA evidence under two hypotheses. To perform this analysis an allele frequency database is required and the most appropriate reference database is the one that best matches the ancestry of the true source of DNA. It is worth noting that the mixed ancestry of the Brazilian population makes more complex to select the correct database. Furthermore, it is also important to consider substructure and inbreeding when performing

the calculations. In this context, this work aims to compare the LRs obtained using the allele frequencies of local populations and the Brazilian population as a single database. For that purpose, we simulated a total of 2,100,000 genetic profiles of 19 Brazilian states and the whole Brazilian population, based on data from literature and assuming Hardy-Weinberg and linkage equilibria. We also computed FIT indexes among the Brazilian population and each Brazilian state. From this dataset, we calculated the LRs with adjusted and non-adjusted formulas, using a perl script. Finally, we performed a Bayesian analysis in the software Structure. Some Brazilian states exhibit significant F values in comparison to the Brazilian population. Our data suggests that the LR values obtained with the allele frequencies of each local population are statistically different from the whole Brazilian population with non-adjusted formulas, but presented a better fit to the LRs calculated with the Brazilian population and the adjusted formulas.

431. EVALUATION OF THE FULL-SIBLING KINSHIP WITH MULTIPLE FULL-SIBLINGS ATTENDANCE

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Inferring kinship is requested in many situations. The exact method and IBS scoring are often used for kinship evaluation in forensics. They are individual-to-individual caparison methods based on statistical data. Here we proposed an approach to use the joint information of known-siblings to evaluate the identity of a suspect, the Multi-participants retro-genetic (MPR) method. The principle of MPR is to use the genetic laws to deduce the possible alleles of offspring from the same ancestor. Since full-sibling relationship is the simplest model for evaluation, we used groups of 2 and 3-known full-siblings to assess the possibility and power of MPR in this paper. STR typing was obtained using Goldeneye™ 20A kit or PowerPlex®16 System containing 19 and 15 STRs information, respectively. The full-sibling relationship was ruled out if 3 or more STRs disobey the genetic laws. When examined with 19 STRs, the exclusion rate was higher than IBS scoring method (95.34% for 2 known full-sibling or 100% for 3 known full-siblings vs. 72.42% with IBS scoring). Those STRs could offer the exclusion allele patterns were named as efficient STR. The exclusion rate increased as the number of efficient STRs grow, but not in a linear manner. The MPR method shows a clarity and high efficiency and may be valuable for future forensic application.

432. FALSE INCLUSION RISK OF DUO PARENTAGE CASES

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DNA analysis is an important tool in missing person identification cases. When direct reference samples are not available, indirect references can be used to identify missing persons. It is possible to compare the questioned sample with different number of relatives, including mother and father. However, under certain circumstances, laboratories may have to perform duo analysis, which can increase the risk of false inclusions. Disregarding the possibility of mutations, in parent-child relationship, at least one allele is shared per locus. Therefore, to evaluate the risk of false inclusions in duo parentage cases, we

simulated 17-loci profiles of 19 Brazilian states and the whole Brazilian population. The simulation was performed using R statistical software and it was based on data from literature, assuming Hardy-Weinberg and linkage equilibria. The profiles of *a priori* unrelated individuals were compared one by one, using an R script. A preliminary analysis of 10,000 profiles simulated from the Rio de Janeiro population shows that 600 pairs of individuals have at least one allele in common in all analyzed loci. These pairs include some profiles with frequent alleles that presented a positive result when compared to more than one individual. The next steps are to extend the analysis to all the simulated profiles and to calculate the likelihood ratio of the cases where allele sharing is observed.

433. FORENSIC STATISTICS ANANLYSIS TOOLBOX (FORSTAT): A STREAMLINED WORKFLOW FOR FORENSIC STATISTICS

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The calculation of summary statistic parameters to describe the usefulness of forensic loci have been conducted extensively using the Powerstats v1.2 tool [1]. As an indication to the relevance of Powerstats v1.2 a Google scholar search on 30 March 2017 indicated 15 citations for 2017. Regardless of this the weblink to this tool was discontinued. Additionally, some of the limitations to using Powerstats v1.2 are: one locus, a maximum of 50 unique alleles, six population groups and 600 individuals per population per excel document.
We have therefore simplified, streamlined and modernized the process by programming the parameter calculations in R v3.3.1 and incorporating several packages [2–6] and the package was coded into HTML5 using shinyR [7]. Our tool utilises the common genepop input format, and calculates allelelic frequencies, MP, DC, PE and PI parameters. The output contains tables and optionally histograms and heatmaps for each population group and locus as per the input file. The analysis of our test file containing 500 individuals typed with STRs [8] was completed in under 6 seconds whereas Powerstats calculations took < 1s for one locus; however, the data has to be collated manually for all loci. Further improvements of the website are under construction.

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435. NEW SOFTWARE AND PROCESS TO RESOLVE COMPLEX DNA MIXTURES

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The evolution in the sensitivity of DNA analysis techniques causes a growth of analyzable DNA

samples. This allows the usage of low-DNA- quantity samples, which often contain DNA from more than one contributor. Resolving these complex DNA mixtures presents a growing analysis challenge for DNA experts. Complicating factors, like degradation, stochastic amplification effects, observable peaks below stochastic and analytical thresholds, and peak height imbalance, necessitate the development of mathematical methods and supporting software capable of advanced mixture interpretation.
During our research and ongoing development of our software GenoProof Mixture we developed a full-continuous model following different approaches. The software includes a full raw data analysis and offers the possibility to analyze your mixed DNA samples with binary and probabilistic models in one analysis process.
The method for calculating the weights of all possible genotype constellations which can explain the given DNA mixture is using a Markov Chain Monte Carlo method. The method considers different parameters like peak heights, stutter-quotient, allele drop - in / drop-out to deliver the best results.
All algorithms were optimized to deliver an excellent run-time behavior also on ordinary desktop computers.
To improve also the quality assurance and the transparency of the mixture analysis we developed graphical tools which facilitate the experts working with complex mixtures clearly, so that they easily can explain the results in court.
We provide insights into our developed model and show examples of how to massively strengthen the effectiveness of complex DNA mixture analysis.

436. SNP ANALYZER – A TOOL TO ANALYZE LARGE SETS OF GENETIC MARKERS ACCOUNTING FOR LINKAGE

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This study presents the development of new software to analyze large sets of genetic markers. In the era of next generation sequencing and high density SNP arrays there is a need for new computer tools to adequately analyze the output. The software in this study relies on the computational core of Merlin, commonly used in medical genetics for linkage analysis (Abecasis, Cherny et al. 2002).
In addition to performing pairwise relationship computations it can be used to conduct simulations to obtain expected values for the likelihood ratios (LR). Simulations will give a general idea to when a case can be solved prior to actually sending samples for analyzes.
The software, implementing an easy interface, will be most useful for labs obtaining data from clients (or directly using) services provided by companies such as 23andMe or FamilyTreeDNA. In addition, it can use population frequency data from the 1000genomes project covering several continents (Consortium 2015). A demo version of our software is available from the author upon request.

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437. STATISTICAL ESTIMATION OF AMBIENT TEMPERATURE FOR MINIMUM POST-MORTEM INTERVAL(PMI) IN KOREA

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Abstract: Estimation of minimum Post-Mortem Interval(PMImin) is a critical issue in Forensic Entomology.[1,2,3] It can be used as evidence in the court to determine exact time of death. Therefore, it is one of the most important factors to estimate the ambient temperature for ADD(Accumulated Degree Day) and PMI in the incident scene. In many studies, the linear regression model has been used and only meteorological temperature is considered as an explanatory variable to estimate relationship between ambient temperature and meteorological temperature. [1,2,3] Until now, the temperature of the nearest weather station was used to estimate the ADD, but it increased the errors between the estimated and real value according to various environmental factors. Therefore, we tried to construct more sophisticated statistical model for estimating past ambient temperature in Korea.

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438. THE INFLUENCE OF THE DIFFERENT MUTATION MODELS IN KINSHIP EVALUATION

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Different mutation models have been developed considering the genotypic observations of parent(s)/child duos (or trios), even though, for autosomal transmission, only Mendelian incompatibilities, not mutations, are able to be identified. The most commonly considered mutation models are the so-called: “equal”, “proportional”, “stepwise” and “extended stepwise”, all implemented in the software familias.

In this work we simulated 100,000 profiles (duos and trios) of parent-child, full-siblings, half-siblings and unrelated individuals, assuming a specific database for 17 autosomal STRs and probabilities of incompatibility inferred from the AABB report, 2008. Using the R version of familias we calculated the likelihood ratio where the probability of the genotypic configuration of the individuals assuming each of the pedigrees was compared with the same probability assuming unrelatedness. In the case of full-siblings, the comparison assuming half-sibship as the alternative pedigree was also considered.

The results show that for profiles generated assuming the above mentioned pedigrees, except for unrelated, the use of different mutation models with parameters inferred from the proportion of observed mendelian incompatibilities does not result in relevant differences, which also indicates that the consideration of hidden mutations does not have a relevant influence in the final result. When the individuals were simulated as unrelated, the mutation models greatly influenced the order of magnitude of the LR.

Future work should be developed to measure the impact for cases where a close relative of the father, such as a brother, is analyzed as the putative father in a standard paternity test.

439. THE INTERPRETATION OF DNA MIXTURES WITH THE PRESENCE OF RELATIVES

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DNA recovered from a crime scene can often be a mixture of more than one individual. The ability to detect a mixture depends on a number of factors. These include the amount and proportion of DNA from each contributor and the number and type of loci analysed. The probability that a mixture will be detected improves as more highly polymorphic loci are typed. Loci that are highly polymorphic have more allele types and therefore there is an increased chance that contributors' profiles will differ at these loci. Mixtures of DNA from relatives tend to have high masking.
The presence of related individuals within a mixture is not uncommon. The high amounts of allele masking mean that interpretation of these mixtures can be challenging.
In this study, three and four person mixtures of varying proportions were artificially generated by mixing DNA from members of different families. The mixtures were amplified using GlobalFiler™ and analyzed on an ABI 3500 capillary electrophoresis instrument. Mixture interpretation was undertaken using a fully continuous probabilistic genotyping method assuming no individuals, conditioning on one individual, and conditioning on one individual with a replicate amplification. The likelihood ratio was calculated to each contributor to the mixture, under these different scenarios. We discuss the results with the view to inform casework policies.

440. THE SIGNIFICANCE OF STATISTICAL PROBABILITY FOR LOCI ABOVE 20

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Forensic DNA analysis are carried out to ascertain the origin of biological sample left at a crime scene to the individuals involved; either the victim, the perpetrator or any other persons. Once there is a match, forensic scientists will calculate the probability of another individual at random having a matching DNA profile to determine the significance of the match. Most forensic laboratory are assessing the DNA profile at 15 STR loci where the probability are around 1015 (quadrillion) – 1021 (sextillion). Since 2013, the forensic community was introduced to the 21 STR loci which increases the probability to 1024 (septillion) – 1030 (nonillion). The increased loci not only enhanced the individualisation of the particular DNA profile but also minimises the adventitious DNA match that may occur. Another factor to consider is that, since the probability is huge; whether we still need to calculate probability based on the ethnicity. At a glance, Malaysia consists of three major ethnic groups, Malays, Chinese and Indians; however, these major groups can be further itemized to numerous smaller ethnicities. Based on the frequencies determined at each of the 21 loci, the probability is still massive even if the highest frequencies of the loci are used to calculate the probability.

441. UNEXPECTED EFFECTS OF MUTATIONS IN KINSHIP TESTING

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Mutations are particularly important for Short Tandem Repeat (STR) markers, where the rate of occurrence may be as high as 1%. In other words, if we consider a duo paternity case with 30 such markers, we could expect to observe an inconsistency in every third case. At this point, it should be noted that not all mutations will lead to genetic inconsistencies and therefore the observed rate will probably be lower.
In this study we investigate some curious effects mutations can have in kinship analysis. Specifically we explore interesting real cases and conceptual cases where these effects are prominent. For instance, we demonstrate that even when two individuals share zero alleles identical by state, the results may speak in favor of relationship. We summarize and emphasize the importance of modeling mutations as well as providing a comparison for some of the commonly used transition models.

442. A MULTI-TISSUE AGE PREDICTION MODEL BASED ON DNA METHYLATION ANALYSIS

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Age related tissue-specific DNA methylation markers have been identified in many forensic studies, and these can be used to estimate the chronological age of an unknown sample donor, which can be exploited for police investigations (1,2). However, in many forensic cases the tissue source of the DNA evidence cannot be identified, and this can result in using an age prediction model for the wrong type of tissue, which in turn may result in an inaccurate age estimation (3). To address this issue, we analyzed >480,000 CpG sites using the Infinium HumanMethylation450 BeadChip (Illumina) in five forensically relevant tissues (whole blood, semen, saliva, menstrual blood, and vaginal secretions) using 41 samples aged between 20 and 59 years. We were able to identify 12 novel CpG sites with significant association with age that can be used to estimate chronological age across the five tissue types. A regression model for multi-tissue age prediction based on the identified CpG markers explained 94% of the variance in age, with an average accuracy of 2.3 years. The model was tested on an independent dataset of 24 samples from four types of tissue (blood, saliva, menstrual blood, vaginal secretions), and the mean absolute deviation from the true chronological age across these tissues was 5.9 years. This study suggests that universal DNA methylation markers will be available in future for age prediction regardless of the tissue type recovered from a crime scene.

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443. A PERSONAL IDENTIFICATION TRIAL WITH POST-SUCKING TIME ESTIMATION FROM HUMAN BLOOD IN MOSQUITOES

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We attempted to estimate the time elapsed since feeding from the degree of human DNA digestion in mosquito blood meals, and to perform personal identification in two species of mosquitoes. After stereomicroscopic observation, extracted DNA samples were quantified using a human DNA quantification and quality control (QC) kit, and were genotyped for 15 STRs using a commercial multiplexing kit. It took about 3 days for complete digestion of a blood meal, and genotyping was possible until 2 days post-feeding. Relative peak heights for 15 STRs and DNA amounts were useful to estimate post-feeding time to about half a day between 0 and 2 days. Furthermore, the quantitative ratios derived from STR peak heights and the QC kit were reasonably effective in estimating approximate PF time, after 2 to 3 days. We suggest that this study may be very useful in estimating time after mosquito feeding from blood meal DNA.

445. AGE-ASSOCIATED DNA METHYLATION DETERMINATION OF SEMEN BY PYROSEQUENCING IN CHINESE HAN POPULATION

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In forensic casework, age-prediction of crime-related biological stains can be of significant importance for the reconstruction of the events at a crime scene. Recently, DNA methylation, patterns in particular age-related CpGs (AR-CpGs) has emerged as an alternative strategy for forensic age estimation, which was reported as tissue specific and showed variant methylation quantitative in different platforms. Semen, as one of the most common biological traces in forensic sexual crime scene, was firstly reported to estimate age based on 3 CpG sites (cg06304190, cg06979108 and cg12837463) with SNaPshot method by Lee et al. In this study, we aimed to evaluate the cg06979108 as potential age prediction marker in semen in Chinese Han population by pyrosequencing approach. To our knowledge, pyrosequencing as a quantitative technique was regarded as the golden standard compared with the SNaPshot method in methylation analysis. A total of 38 unrelated male volunteers with ages from 21 to

54 years were successfully analyzed. We fit a linear regression model for age prediction with R^2 = 0.739. We validated the linear regression model with other 17 samples, and the prediction of the mean absolute deviation (MAD) was 4.048 years.

446. AN INTEGRATED ANALYSIS TOOLS FOR CPG ISLAND PREDICTION AND PRIMER DESIGN

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DNA methylation plays an important role not only in epigenetics such as gene expression regulation but also the in forensic science for age estimation studies and body fluid identification. The DNA methylation occurs mainly in the CpG site in the genome, and the high frequency portion of the CpG dinucleotide and G-C contents is called the CpG islands. Programs for finding CpG islands have continually been developed. However, most programs only provide structural information for the predicted CpG islands, and no experimental information is available to confirm the methylation of the CpG site. In this study, we developed an analysis tool package for predicting CpG islands by sliding-window method and designing primer sequence based on predicted location. The primer designing programs are separated into standard PCR and bisulfite PCR programs according to bisulfite treatment of DNA, respectively. Furthermore, a methylation-specific PCR (MSP) program was developed to identify the methylation of particular CpG sites. We tested the performance of this program by finding the CpG islands with the hg38 reference sequence. And then we also experimentally verified the primers designed based on the location of predicted CpG islands. This program can be a useful tool to find novel target CpG islands and methylated-CpG sites, and it can be applicable in various fields of DNA methylation research as well as in epigenetics and forensic science.

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447. ASSIGNING PHENOTYPIC AND ANCESTRY INFORMATION TO VICTORIA'S UNIDENTIFIED HUMAN REMAINS – A PILOT STUDY

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Current investigation of missing persons in Victoria incorporates a multi-disciplinary approach. The ante-mortem information combines personal information and description of the missing person, as well as any dental and medical records, and where possible, fingerprint information. Victoria currently utilises both nuclear DNA analysis (via STR multiplex) and mitochondrial DNA (via Sanger sequencing) to perform kinship and direct comparisons to reference profiles. Most unidentified remains are discovered

in a state of decomposition, skeletisation and/or incomplete, limiting information generated from the post-mortem examination and investigative leads. In the absences of physical description, DNA analysis is a powerful investigative tool for establishing identity; however it is limited by the ability to establish suitable references through suspicion of identity or the size of the reference database available for searching. The expanding body of work on phenotypic and ancestry SNPs, and growing acceptance of Massively Parallel Sequencing in forensic science, will soon see port-mortem reports for unknown deceased containing information mirroring the personal descriptors in missing persons reports – such as hair and eye colour and ancestry. This study details the application of the Ion AmpliSeq™ Precision ID Panel (Thermo Fisher) on 31 unidentified remains cases, with the aim to assess the phenotypic and ancestry inferences, as well as the effectiveness of the AmpliSeq™ Precision ID Panel and Ion Torrent workflow on degraded samples. Using the phenotypic predictive tool Hirisplex and the Iron Torrent analysis software (Thermo Fisher), phenotypic and/or bio-geographical results have been obtained for 27 cases.

448. AUTOSOMAL AND Y-STR: A COMBINATORY APPROACH TO ETHNICITY INFERENCE IN SINGAPORE

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Autosomal Small Tandem Repeat(STR) loci are ideal for human identification due to their high mutation rate which results in high level of polymorphism observed across individuals. Other than identification, it has been reported that ethnicity inference may be possible through consideration of ethnic-specific allele frequency distributions [1-5]. Y-STR loci, on the other hand, have lower power of discrimination compared to autosomal STR loci as they are passed down the males within the same paternal lineage without any recombination [6]. However, it has been previously shown that Y haplotypes can be clustered according to ethnic groups, a model that can predict the ethnicity of a male DNA profile with high confidence [7]. Singapore is a metropolitan country with her native population made up of three major ethnic groups, namely, Chinese, Malay and Indian, in different proportions. We have previously established ethnic-specific allele frequencies for all three ethnic groups [8-10]. In the present study, unrelated male samples, previously processed with GlobalFiler™ Express amplification kit, were processed with the Y-Filer Plus amplification kit, after which Y haplotype and haplogroup frequencies were generated. By combining the autosomal and Y-STR information, we investigated the development of an inference tool to provide insights as to the ethnicity of the male donor of the DNA recovered from a crime scene sample.

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449. BMI-ASSOCIATED SNP GENOTYPES AND BODY SHAPE PREDICTION IN A KOREAN POPULATION

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Forensic DNA phenotyping aims to infer the appearance of an unknown sample donor from DNA. When there is no suspect to match, or the DNA profile from the evidence does not match anyone in the forensic database, forensic DNA phenotyping is expected to provide an investigative lead that can facilitate a traced search for an unknown suspect by narrowing the search range. Other than pigmentation SNPs and epigenetic age signatures, body mass index (BMI)-related markers are also expected to be useful in inferring the appearance of donors. A recent genome-wide association study has identified 55 genetic loci associated with obesity or BMI among 86,757 individuals of Asian ancestry. Among these loci, 51 had been identified in European ancestry, and 8 loci were significant at $P < 5E-8$ in Asian populations as well as European populations. In this study, we designed a multiplex SNaPshot reaction to score these 8 SNPs (rs1558902, rs11030104, rs574367, rs591166, rs12463617, rs11671664, rs6545814, and rs16858082 near the genes, *FTO, BDNF, SEC16B, MC4R, TMEM18, GIPR/QPCTL, ADCY3/RBJ* and *GNPDA2*, respectively), and analyzed genotypes of 700 Koreans with varying range of BMI. Based on the genotype results and BMI, we evaluated the practical applicability of these SNPs to the prediction of body shape or BMI for investigative leads. DNA samples were obtained from voluntary donors or from bioresources of National Biobank of Korea, the Centers for Disease Control and Prevention, Republic of Korea (2016-067).

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450. CASEWORK EXPERIENCES WITH DETAILED SNP-BASED ANCESTRY ANALYSES USING ION TORRENT MASSIVELY PARALLEL SEQUENCING

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We have begun to apply massively parallel sequencing (MPS) to a range of forensic investigations by genotyping several new and established marker sets using this technology. Amongst these, SNP-based ancestry tests are in the early stages of adoption by the forensic community as a way to obtain investigative leads or improve identification of the remains of missing persons. We report forensic casework experiences applying a commercial 165-SNP ancestry panel from Thermo Fisher; an in-

house 127-SNP AmpliSeq-primer ancestry panel designed to address the problem of population admixture [1]; and a newly designed 165-marker AmpliSeq-primer ancestry panel, which adds multiple-allele SNPs and microhaplotypes [2] to binary SNPs. We have made extensive use of genetic data analysis regimes previously developed for capillary electrophoresis-based SNP analysis, including Bayes analysis and PCA in the *Snipper* suite (http://mathgene.usc.es/snipper/), and STRUCTURE/ADMIXTURE genetic cluster detection algorithms. All these systems of analysis have been relatively easy to adapt to routine casework and they provide flexible approaches to each case's analysis requirements, such as: partial profile data; incomplete reference SNP genotypes; differentiation of closely related population groups; or individual co-ancestry from admixture. We are currently adapting these regimes to analyse microhaplotype data alongside SNP genotypes and report this work, as well as examples of casework ancestry analyses that used the three MPS ancestry marker sets individually or in combination.

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2. Kidd K, et al, Evaluating 130 Microhaplotypes across a Global Set of 83 Populations, Forensic Sci. Int. Genet. (2017); accepted for publication.

451. CHARACTERISTICS OF SNPS RELATED WITH HIGH MYOPIA TRAITS IN CHINESE HAN POPULATION

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High myopia, as a kind of phenotypic traits, can be used to enhance the ability of personal identification in forensic case. To elucidate single nucleotide polymorphisms (SNPs) of microRNA related with high myopia in Chinese Han population, five SNPs were investigated with 200 (100 high myopia and 100 normal subjects) unrelated individuals from Sichuan province through SNaPshot method. Two SNPs showed a significantly different (p<0.05) allele-frequency distribution between high myopia and normal subjects. There were no statistical significant differences at other three SNPs. According to this primary research, the genotype frequency for two SNPs (rs8004825 and rs12976445) did correlate with high myopia phenotype. To predict high myopia with high accuracy, more samples and genetic markers should be performed in further study.

452. COMPARISON OF ANCESTRY ESTIMATION STRATEGIES USING DIFFERENT MARKER SETS

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Ancestry informative markers (AIMs) have been used in forensic genetics to infer biogeographic ancestry of an individual and may also have a prominent role in future police investigations.
Frequently used metrics proposed for AIM selection rely on maximization of genetic distances. One of the major setbacks of this method is maximization of the error associated with each analysis. Large genetic distances are usually connected with strong drift and/or selective pressure and, therefore, ancestry inferences using these markers can be highly influenced by the correct definition of contributing (reference) populations.
In this work, we propose instead to assess the suitability of ancestry inference of each marker by studying families with mixed parentage and their offspring. Since full-siblings have the same apportionment of common ancestry inherited from their parents, the most informative loci will be those presenting the smallest degree of deviation between the observed and expected ancestry proportions.
We report data for 32 Brazilian families with two children and parents of different ancestry.
Individual ancestry was investigated with two different AIM panels: 46 indels (Pereira et al. 2012; PLoS One 7:e29684) typed with PCR and capillary electrophoresis and 165 SNPs (the Precision ID Ancestry panel, Thermo Fisher Scientific) typed with massive parallel sequencing.
Different pairwise comparisons of ancestry estimates between the family members were studied. The performances of the two panels were compared. The best combination and minimum number of markers that better reflected the true ancestry proportions of the full-siblings were addressed.

453. CORRELATING DNA METHYLATION DATA WITH CHRONOLOGICAL AGE: A QUEST FOR THE OPTIMAL STATISTICAL MODEL

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Forensic investigations can benefit substantially from intelligence information, and the accurate estimation of an individual's age has the potential to serve as a great asset in numerous scenarios. Approaching ageing as a biological phenomenon that occurs in part through differentiation in gene expression, the search for age predictors has focused on epigenetic factors as these are known gene modulators. Amongst them, DNA methylation has been proven to correlate strongly with age, with recent projects yielding numerous methods for DNA methylation based age prediction. However, little research has so far been carried out on the computational/statistical approaches that can be employed when correlating methylation values with chronological age. This study investigates the advantages and disadvantages of various statistical modelling methods when used in DNA methylation based age prediction. From simple linear models, to support vector machines and the highly sophisticated modelling of artificial neural networks we have taken a closer look at the statistics in order to find out if there is, indeed, a statistical model that can make the most of every methylation dataset. These models have been tested on a DNA methylation based age prediction method developed in whole blood using 12 age-associated CpG sites.

454. DEVELOPMENT OF A NEW AGE PREDICTION ASSAY USING THE MS-SNUPE STRATEGY FOR FORENSIC

PURPOSES

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Age estimation of a biological sample using methylated DNA analysis has raised great interest in the forensic field in recent years. When no information of the donor of a sample is available, the prediction of age could guide police investigations, for either the search of possible suspects or victim identification, and it can also potentially improve the assessment of age-related phenotype patterns. Recently, we have published a set of CpG positions correlated with chronological age [1] that allows forensic age prediction with one of the lowest predictive error ranges published to date. However, the methylation analysis strategy, despite being robust and informative as a discovery tool, is not suitable for forensic requirements, especially in terms of the minimum levels of DNA and equipment needed. Here we present a new assay that includes the previous CpG set plus the evaluation of new methylation markers. The new strategy is based on a methylation-sensitive single nucleotide primer extension (MS-SNuPe) approach. Briefly, a DNA bisulfite converted sample presents C or T at CpG sites depending on the methylation state (C if the CpG site is methylated, T if non-methylated) and nucleotides are analysed with a Single Base Extension sequencing method (SBE). We present the results of the assay in samples of different ages. The methylation patterns obtained were used to develop a predictive model to estimate the age of the sample donor. This strategy provides a better option for the implementation of a sensitive age prediction test for routine forensic use.

1. Freire-Aradas, A., et al., *Development of a methylation marker set for forensic age estimation using analysis of public methylation data and the Agena Bioscience EpiTYPER system.* Forensic Sci Int Genet, 2016. 24: p. 65-74.

455. EVALUATION OF CLOCK GENES EXPRESSION PATTERNS FOR PREDICTION OF THE TIME-OF-DEPOSITION OF A BIOLOGICAL SAMPLE

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During police investigation of a crime, every additional piece of information can be precious. The determination of the time-of-day when the sample was deposited could guide many investigations with potentially valuable information about activity level suppositions. To know the time-of-deposition of a sample could help to exclude suspects that had not been at the crime scene within a given timeframe or could be indicative of the time the crime was committed. We have approached the prediction of time-of-deposition by studying Clock genes that play a core role in the regulation of circadian rhythms, which lead to metabolic events that oscillate across the diurnal timeframe. The selection criteria for the gene set under study, including a total of 9 Clock genes, suggested to be involved or related to circadian rhythm control and suspected cyclical expression of proteins during 24 hour cycles. Both blood and

saliva samples were collected at different times of the day, and the corresponding expression levels of mRNA were quantitatively measured by real-time PCR. Differences in gene expression levels at different time points in 24 hour cycles were compared and the most informative markers were selected for further research and forensic test development.

456. EVALUATION OF DNA METHYLATION-BASED AGE PREDICTION ON BLOODSTAIN SAMPLES

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In recent years, DNA methylation analyses has been shown to be the most promising methodology in the area of forensic age prediction. In our study, using only 3 CpG sites, the methylation level of the DNA samples extracted from liquid blood were measured using bisulfite pyrosequencing. The age prediction model developed herein had a validated mean absolute deviation of 5 years and a prediction error of 6.1 years. However, the large amount of DNA, 500 ng, required for bisulfite conversion would impede the application of this age prediction method on crime samples. In addition, there is limited information on whether age prediction based on DNA methylation can be performed on samples commonly recovered from a crime scene. Hence, this study also evaluated the robustness of the age prediction methodology using only 50 ng of DNA samples extracted from blood stains on swabs and clothing. The reduction in DNA template requirement will strengthen the application of methylation-based age prediction in the forensic context.

457. FORENSIC AGE ESTIMATION TESTS EXTENDED TO DNA METHYLATION PATTERNS IN CHILDREN AND ADOLESCENTS

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Since DNA methylation levels are observed to gradually change during an individual´s lifetime, several forensic age prediction models have recently been introduced, the majority based on blood samples covering adult age ranges. To date, children and/or adolescent age ranges have not been fully represented in published forensic age predictors. There is evidence that alterations to DNA methylation patterns during early periods of life occur at a higher rate compared to adult patterns. A robust system able to accurately predict individual age in children and adolescents as well as adults could be useful to guide human identification of missing persons or disaster victims as well as providing valuable investigative leads from contact traces. Additionally, legal disputes could be supported by the inference of individual age to ascertain if an official procedure should be applied to an older juvenile or to an adult. In this study, we have investigated DNA methylation of eight genes across the human genome in blood DNA samples from subjects between 3 and 17 years old. DNA methylation levels were obtained using Agena EpiTYPER MALDI-TOF based CpG analyses, as previously applied in a recently published age prediction model [1]. Differences in DNA methylation levels observed versus chronological age were

assessed in order to select the most informative CpG sites. Subsequently, an age prediction model has been developed.

1. Freire-Aradas A, Phillips C, Mosquera-Miguel A, Girón-Santamaría L, et al., Development of a methylation marker set for forensic age estimation using analysis of public methylation data and the Agena Bioscience EpiTYPER system. Forensic Sci Int Genet. 2016, 24: 65-74.

458. FORENSIC AGE PREDICTION MODELS: ADAPTING DNA METHYLATION DATA FROM PYROSEQUENCING TESTS TO A QUANTILE REGRESSION MODEL

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Several forensic age prediction models based on DNA methylation data have recently been developed, most making use of linear regression analysis (LR). LR is an approach for modelling the relationship between interrogated variables (chronological age) and one or more observed variables (DNA methylation levels from multiple CpG sites). Therefore, LR is a useful method for forensic age estimation using methylation levels estimated from a selected set of CpG sites from the most age-correlated genes. However, LR has the characteristic of establishing a fixed predictive interval for the full age range of the subjects used to construct the model. Additionally, when methylation levels of older subjects are analysed, higher errors are always obtained. This characteristic is known as heteroscedasticity and has been detected in most forensic age prediction systems. One way to solve this issue is calculation of specific errors for different age ranges. Quantile regression (QR) is an alternative prediction method to LR permitting the calculation of a predictive interval for a given set of age ranges, each of which can be relatively narrow. In this study, we assessed an established LR model based on pyrosequencing data [1] when applying a quantile regression analysis module we developed for EpiTYPER data and made accessible through the Snipper forensic age estimation portal [2]. More than 300 blood-based DNA samples were used for the fresh analyses. The accuracy of age inferences using quantile regression and their predictive intervals were evaluated and compared with LR inferences for each sample of known age.

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2. Freire-Aradas A, Phillips C, Mosquera-Miguel A, Girón-Santamaría L, et al., Development of a methylation marker set for forensic age estimation using analysis of public methylation data and the Agena Bioscience EpiTYPER system. Forensic Sci Int Genet. 2016; 24: 65-74.

459. GENOTYPING OF ELEVEN SNPS ASSOCIATED WITH FACIAL HAIR IN CHINESE HAN POPULATION

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DNA-based prediction of externally visible characteristics (EVCs) of an unknown person who left a DNA sample at a crime scene is expected to be useful for police investigation if no direct suspects can be identified by conventional means of investigations. A recent Genome-wide association studies reported novel genetic variants in Latin Americans for features of facial hair (1). Our study aimed to investigate the associations between eleven SNPs and facial hair in a Chinese Han population. Our study consists of 420 volunteers. Genomic DNA was extracted using a Genomic DNA life kit by BIOTEKE CORPORATION. Genotyping of these SNPs was carried out by the PCR-RFLP methods, the Snapshot methods and the direct sequencing methods. A significantly associations was observed in the rs1868245 GG genotype compared with the GT or TT genotypes (GG versus GT/TT: OR = 1.61, 95% CI = 1.02-2.54, P values = 0.042), and the rs10908366 TT genotype compared with the CC genotype (TT versus CC: OR = 0.53, 95% CI = 0.29-0.97, P values = 0.039). Our result suggest that, the rs1868245 and rs10908366 variants may be able to be used as markers for predicting human facial hair in Chinese Han population.

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460. HELPING THE IDENTIFICATION OF REFUGEE SHIPWRECK VICTIMS IN THE STRAITS OF SICILY: AN AIM-INDEL REFERENCE DATABASE FOR THE TIGRAY POPULATION OF ETHIOPIA

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In mass disasters involving subjects of several nationalities, ancestry informative markers (AIMs) can contribute to the identification process by: 1) enabling the matching of human remains and reference samples from an unknown person's relatives according to their ancestry; 2) guiding the choice of proper reference STR frequency databases for identity/kinship calculations.
The refugee crisis that hit Europe in the 2010s was marked by many tragedies. One of the worst accidents occurred on 18th April 2015, when a migrant vessel sank in the Straits of Sicily, with over 800 deaths. According to UNHCR, people from Syria, West and East Africa (including about 350 Eritreans) were on board [1]. In 2016, the Italian Government launched a task force to raise the sunken boat, perform autopsies on recovered bodies, and collect samples for future DNA testing [2].
To anticipate future DNA analyses, a reference population dataset of 46 AIM-Indels (n=228) was obtained from the Tigray population (the major ethnic group in Eritrea and Northern Ethiopia). The AIM-indel panel was highly effective in discriminating between Ethiopian Tigray and other sub-Saharan

African populations, including populations of the HGDP-CEPH panel. However, considerable genetic overlap exists between the Ethiopian Tigray sample and HGDP-CEPH Middle Eastern populations. These results, as well as data collected from post-mortem examinations, will contribute to an improved identification process of deceased migrants from East Africa recovered from the Mediterranean Sea.

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461. IMPACT OF GENETIC ANCESTRY ON CHRONOLOGICAL AGE PREDICTION USING DNA METHYLATION ANALYSIS

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Recently, several forensic age predictors based on DNA methylation have been developed, reporting a considerable number of CpG loci highly correlated with the chronological age. Most of the published age prediction models so far were based on European samples, and therefore there is a need to explore additional worldwide populations. DNA methylation patterns may be altered if individuals are exposed to various environmental factors or have different biogeographic ancestries. Therefore, different populations around the globe should be examined. To this end, we performed a comprehensive analysis of methylation profiles from buccal mucosa samples of four independent population groups from Middle East, West Africa, East Africa and Central Europe. For each population group approx. 50 samples with an equal distribution of donor ages were collected. Using bisulfite pyrosequencing we analyzed the methylation patterns at six CpG loci in the in four genes of *ASPA*, *ITGA2B*, *PDE4C* and *ELOVL2* that are already known to be suitable age-predictive markers. Age estimations were done using two previously reported age prediction models (Eipel et al., Aging 2016, 8:1034-1048; Zbieć-Piekarska et al., 2015, FSI Genet. 14:161). Differences between epigenetic and chronological age were assessed and discussed in the four population groups.

462. IMPROVING PREDICTION OF PIGMENTATION TRAITS BY QUANTITATIVE PHENOTYPE EVALUATION AND MASSIVELY PARALLEL SEQUENCING

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Tremendous progress in prediction of human pigmentation traits has been made over the last few years. Available prediction models demonstrate a high accuracy of prediction of some phenotype categories, but explaining the missing heritability is crucial for better prediction of some pigmentation categories like intermediate eye colors or particular skin shades observed in European populations. Some age-related phenomena like hair darkening around puberty and hair greying remain unsolved. Furthermore, the effects of gender and population of origin should be more explored to fully understand determination of human pigmentation. Also, quantitative evaluation of eye, hair and skin colour may provide better insight into the genetics of these traits.
Here we present results of the project NEXT, which assumes developing predictive DNA system for human appearance involving massively parallel sequencing and innovatory prediction modeling. In the study, 700 samples from Poland were analyzed using Ion AmpliSeq™ technology and Ion S5™ system covering 75 candidate genetic variants for human pigmentation traits. The phenotypic regime involved quantitative measurement methods including spectrophotometry for hair and skin colour and high-resolution photography for iris colour. The information considered also involved gender, age, population of origin, presence of hair darkening in childhood and premature hair greying. The study reports findings from association and prediction analyses with special focus on pigmentation categories predicted with the lowest accuracies and a potential influence of age and gender on prediction results.
The study was funded by the project NEXT supported by the National Centre of Research and Development in Poland, no. DOB-BIO7/17/01/2015.

463. LIKELIHOOD RATIO AND POSTERIOR ODDS IN FORENSIC GENETICS: TWO SIDES OF THE SAME COIN – WITH APPLICATION TO FORENSIC DNA PHENOTYPING

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It has become widely accepted in forensics that, owing to a lack of sensible priors, the evidential value of matching DNA profiles in trace donor identification or kinship analysis is most sensibly communicated in the form of a likelihood ratio (LR). This restraint does not abate the fact that the posterior odds (PO) would be the preferred basis for returning a verdict. A completely different situation holds for Forensic DNA Phenotyping (FDP), which is aimed at predicting externally visible characteristics (EVCs) of a trace donor from DNA left behind at the crime scene. The statistical models underlying FDP typically yield posterior odds (PO) for an individual possessing a certain EVC. This apparent discrepancy has led to confusion as to when LR or PO is the appropriate outcome of forensic DNA analysis to be communicated. We thus set out to clarify the distinction between LR and PO in the context of forensic DNA profiling and FDP from a statistical point of view. In so doing, we also addressed the influence of population affiliation on LR and PO. In contrast to the well-known population dependency of the LR in DNA profiling, the PO as obtained in FDP may be widely population-independent. The actual degree of independence, however, is a matter of (i) how much of the causality of the respective EVC is captured by the genetic markers used for FDP and (ii) by the extent to which non-genetic such as environmental causal factors of the same EVC are distributed equally throughout populations.

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464. META-ANALYSIS OF GENOME-WIDE ASSOCIATION STUDIES IDENTIFY 9 NOVEL LOCI INVOLVED IN HUMAN HAIR CURLINESS

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Hair shape is an externally visible trait potentially useful in forensic DNA phenotyping. We performed a set of GWASs and replication studies in a total of 28,701 subjects from 9 cohorts with multiple ethnic origins. A meta-analysis of three European GWASs identified 8 novel loci (1p36.23 *ERRFI1*/*SLC45A1*, 1p36.22 *PEX14*, 1p36.13 *PADI3*, 2p13.3 *TGFA*, 11p14.1 *LGR4*, 12q13.13 *CALCOCO1*, 17q21.2 *KRTAP*, and 20q13.33 *PTK6*) and confirmed 4 previously known loci (1q21.3 *TCHH*/*TCHHL1*, 2q35 *WNT10A*, 4q21.21 *FRAS1*, and 10p14 *LINC00708*/*GATA3*) showing genome-wide significant association with hair curliness. All except 1p36.22 *PEX14* were replicated in at least one of the 6 additional cohorts with nominally significant evidence. Association testing for 8 previously reported hair curliness SNPs further confirmed *EDAR*, *OFCC1*, and *PRSS53* genes being involved in hair curliness. A candidate gene study of keratin-related genes identified an additional novel locus at 12q13.13 *KRT82*, which is about 1.3 MBp downstream of the *CALCOCO1* locus. A multivariable analysis demonstrated that 13 SNPs in 13 genes independently contribute to hair curliness variation. A prediction model including 51 SNPs provided a substantially improved accuracy compared to a previous model, reaching an AUC value of 0.71. The pattern of the predicted curliness for 2,504 subjects from the 1000-genomes panel is largely consistent with the known global distribution of hair curliness. In summary, the 9 novel loci identified here as being involved in human hair curliness variation substantially improve the genetic knowledge of this externally visible trait and may serve as guides for future studies exploring potential applications in forensics.

465. MORPHOMETRICS, CRANIOFACIAL DISEASE GENES, AND THE QUEST FOR THE GENETIC BASIS OF FACIAL MORPHOLOGY

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This research introduces a low-cost method for identification of genes that may prove useful for prediction of facial morphology from DNA. This prediction capability would provide a novel way to exploit DNA samples from unknown remains or that are found at crime scenes and do not result in database hits.
Candidate genes for facial morphology were identified from genes implicated in craniofacial disorders and selected single nucleotide polymorphisms (SNPs) within them were genotyped within a normal, European population. Facial morphology was captured from landmarks mapped onto 2D photographs. Variation in distance between pairs of landmarks was analysed as a function of genotype for each SNP. Results showed that specific inter-landmark distances varied significantly (alpha = .05) between genotype groups for two of the SNPs investigated, indicating these genes may influence those aspects of facial morphology. The first of these, located in the POLR1C gene, indicated an effect on several measurements of the mid and lower face. The second, located on the LMNA gene, indicated an effect on measurements of the midface around the nose. In both cases the affected region fit with the phenotype of the associated craniofacial disorder.
It was concluded that the POLR1C gene may play a role in the development of bones of the mid and lower face and the LMNA gene may have some effect on morphology of the midface, specifically the nasal region. A more complete understanding of facial genotype-phenotype relationships could form the basis for accurate facial predictions.

466. PHENOTYPING THE ANCIENT WORLD: THE PHYSICAL APPEARANCE AND ANCESTRY OF VERY DEGRADED SAMPLES FROM A CHALCOLITHIC HUMAN REMAINS

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In many cases, the genetic study of ancient samples is quite similar to a forensic critical sample analysis with an unknown origin. In both cases, it is not possible to compare the genetic information with other family members, being almost impossible to achieve the individual identification. In such

situations, the prediction of externally visible characteristics (EVC) of an individual and his biogeographical ancestry could definitely be a crucial contribution in a forensic casework.
Therefore, the aim of the present work was the molecular study of a very critical sample, a Chalcolithic (3480±30 YBP) individual found in Asturias, Northern Iberia, intending to discover a possible geographical ancestry (1) for these remains, and the inference of a group of feasible EVCs (hair, skin and iris pigmentation) (2).
Given that ancient DNA is often highly damaged, two different methodologies were used in order to determine the biogeographical ancestry of the individual: mitochondrial DNA (HVR-I and -II) and Single Nucleotide Polymorphisms (1) typing, since only short fragments of target DNA are required for a successful amplification.
Despite the antiquity of the samples, the genetic information recovered proved of great value. We could determine that the samples donor had a European ancestry, blond hair, light skin color and brown eyes. Such outcomes reveal that it is possible to obtain not only biogeographical but also phenotypic information from a very critical sample.

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467. POTENTIAL FORENSIC APPLICATION OF DNA METHYLATION PROFILING FOR IDENTICAL TWIN IDENTIFICATION

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In a forensic field, short tandem repeat (STR) markers are useful in identifying individuals found at crime scenes, but they can't be used to distinguish genetically identical monozygotic (MZ) twins. This inability causes problems in criminal or paternity cases with a MZ twin as suspect, or as alleged father. DNA methylation, which occurs at the 5'-position of cytosine in CpG dinucleotides, is a genetically programmed type of DNA modification in mammals. Recent studies have shown that monozygotic twins are epigenetically indistinguishable during the early years of life, but older individuals present significant differences in their overall content and genomic distribution of DNA methylation. Therefore, epigenetic changes such as DNA methylation or histone modifications have a great potential to be used for discriminating MZ twins. Here, we investigated genome-wide differences in DNA methylation among 12 paired MZ twins using Illumina's Human Methylation 450K array. From more than 480,000 CpG sites, we selected several hundreds of differentially methylated regions (DMRs) for each twin pair. Next, we selected candidates by searching for recurrent DMRs among the 12 paired MZ twins. Some preliminary data will be shown in here.

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468.PREDICTION OF ADMIXED BIOGEOGRAPHICAL ANCESTRY

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In the absence of DNA profile matches between biological evidence, suspect reference samples and DNA database records, forensic investigations have relied on other forms of evidence to identify perpetrators of a crime, including eyewitness testimonies. However, these are highly susceptible to false memories and bias. Phenotype prediction can provide forensic intelligence by giving insight into the suspect's biogeographical ancestry (BGA). Colonialism and migration have led to widespread admixture and the ability to accurately determine BGA admixture in unknown DNA donors is essential. Pritchard's STRUCTURE[1] program has been considered the gold standard for population analysis, and its ability to accurately predict BGA from genotype has previously been compared with a generic Bayesian algorithm, multinomial logistic regression (MLR) and a novel genetic distance algorithm (GDA) for non-admixed individuals[2]. The current work assesses the ability of these algorithms to classify artificially admixed individuals from Africa, Europe, East Asia, and America. The results show that STRUCTURE and our GDA are most accurate for predicting admixture. Our GDA has the advantage of not requiring long simulation run times or strict formatting of input files. Identification of ancestral population "poles" further reduces complexity of obtaining training samples. Finally, development of a novel graphical representation of ancestry will also be addressed.

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469. PREDICTION OF BIOGEOGRAPHICAL ANCESTRY BY A HYPOTHESIS TESTING APPROACH WITH GENOGEOGRAPHER

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We present a case with an unidentified body where prediction of biogeographic ancestry assisted the investigation. A sample from the body of the unidentified individual was typed for 165 ancestry informative markers (AIMs) using the Precision ID Ancestry Panel (PAP - Thermo Fisher Scientific) and massively parallel sequencing. The resulting AIMs-profile was investigated by a hypothesis testing

approach with our recently developed software GenoGeographer. Using the presently available published data with PAP and GenoGeographer, individuals can be assigned to belong to one of four statistically different clusters representing the sub-Saharan African, Eurasian, Asian, and Somali population groups ($P_{rejection} > 0.05$). The GenoGeographer software first tests if the investigated AIMs-profile can be grouped together with at least one of the populations in the database ($P_{rejection} > 0.05$). Thereafter, GenoGeographer calculates the likelihoods of the AIMs-profile in the various populations in the databases, and sort the likelihoods according to the values of the likelihoods. For the investigated unidentified body, the maximal likelihood of the AIMs-profile among the four clusters was obtained with AIMs data of Eurasian individuals ($P < 0.05$; all LRs >1,000,000 when comparing with sub-Saharan African, Asian, and Somali individuals). When the AIMs-profile of the unidentified body was compared with all populations in the database, the maximal likelihood was obtained when comparing with Moroccan individuals. However, there was no significant difference between the likelihoods obtained with Moroccans and other Eurasian groups in the database ($P > 0.05$).

470. PREDICTION OF EXTERNALLY VISIBLE CHARACTERISTICS OF A PERSON FROM A DNA SAMPLE USING DEEP MACHINE LEARNING APPROACH

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Forensic Molecular Phenotyping (FMP) is a relatively new discipline aiming to predict external appearance and biogeographic ancestry of a person from a DNA sample. The ability to provide such investigative leads offers an unprecedented advance in combating terrorism, disaster victim identification and criminal investigations.
Recent publications by several research groups identified a number of genes and SNPs as potential candidates for affecting normal craniofacial appearance and suggested that it is possible to approximately reconstruct salient aspects of the visual appearance of an individual from partial genetic data [1-4]. Furthermore, latest advances in machine learning have demonstrated that is possible to learn multi-factorial relationships between inputs and outputs with complex structure, similar to facial morphology [].
We have designed a software pipeline combining Artificial Neural Networks (ANNs) and Deep Convolutional Neural Networks (DCNNs) to learn a function that estimates visual appearance from DNA. We have used a dataset of 3,300 single nucleotide polymorphisms (SNPs) and 104 phenotypic traits in 587 DNA samples to train the Neural Networks Pipeline (NNP). The function has been developed and evaluated using a holdout and cross-validation methods. The key measure of performance was the accuracy of the estimations. The preliminary outcome of this study would be presented.
The results of this study provide an important milestone towards development of a comprehensive approach for prediction of externally visible traits and ancestry from an unknown DNA sample.

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471. PREDICTIVE DNA ANALYSIS BY ON-ARRAY MINISEQUENCING IN DNA IMAGING TECHNOLOGY

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Predictive DNA Analysis have been included as standard forensic analyses. Several SNP markers linked with phenotypic traits were have already been described. The most well-known markers include those associated with the iris pigmentation, hair and skin colour as well as blood types in the AB0 system.
The method includes a multiplex PCR amplification of 24 SNP loci associated with pigmentation and 5 associated with the AB0 group system: N29insA, rs11547464, rs885479, rs1805008, rs1805005, rs1805006, rs1805007, rs1805009, Y152OCH, rs2228479, rs1110400, rs28777, rs16891982, rs12821256, rs4959270, rs12203592, rs1042602, rs1800407, rs2402130, rs12913832, rs2378249, rs12896399, rs1393350, rs683, AB0-SNP1, AB0-SNP2, AB0-SNP3, AB0-SNP4, AB0-SNP5. The fragments are amplified in a single PCR reaction, purified and then subjected to cyclic SBE reaction of oligonucleotides immobilized on a array in a Mastercycler Nexus Flat (Eppendorf) thermocycler, then rinsed two times in deionized water in a High Throughput Wash Station (ArrayIt), dried by centrifugation in a Microarray High Speed Centrifuge and analysed using a GenePix4300A (Molecular Devices) scanner at 4 different ranges of excitation and cut-off lengths for specific dideoxynucleotides.
Due to the use of cyclic matrix mini-sequencing the sensitivity and specificity of the reaction is notably enhanced. The quantification level is at 0.01ng/µl. The use of a matrix divided into 4 sub-matrices dedicated to the analysis of positive and negative control as well as two analyses of target sample allowed to conduct the typing of 29 predictive markers during a single analysis with ensured control of highest test quality.

472. PREMATURE HAIR GREYING IN YOUNG PEOPLE AND AGE ASSOCIATED DNA METHYLATION DIFFERENCES BASED ON 5 AGE RELATED MARKERS

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Based on the literature review it seems that implementation of the new technics like massively parallel

sequencing (MPS) might allow to perform effective forensic DNA phenotyping from the samples collected at the crime scene. Prediction models of human hair, skin and iris pigmentation based on single nucleotide polymorphism are developed and available in commercial kits. Furthermore few age-prediction models based on the measurement of DNA methylation level are also developed. The links between human appearance, age, DNA variants and epigenetic changes are not clear, thus further research in that field is still required. Some aspects of human appearance like gray hair or baldness may suggest the age of the individual. Gray haired people are perceived as older than they are. Differentiation between chronological and perceived age by eye-witness in forensic cases may lead authorities to confusion. To mitigate this limitation, we decided to implement 5 age-correlated markers: ELOVL2 c7, C1orf123 c1, FHL2 c2, TRIM59 c7, KLF14 c1 revealed in our laboratory, to test methylation level of 100 samples from gray haired young people and 100 controls (same age and sex). In this experiment converted DNA (bisulfide treatment) was amplified using primers designed in previous study. Pyrosequencing was performed using Pyro Gold reagents on a PyroMark Q24 instrument with workstation. Data were automatically analyzed using PyroMark analysis software (Qiagen, Hilden, Germany). The results obtained in the study allowed verification of the biological age of the tested samples and correlation between hair graying process and age.

473. SEQUENCING OF THE HERC2-OCA2 REGION IN RELATION TO HUMAN EYE COLOURS

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Human eye colour can for the most part be explained by a single nucleotide polymorphism (SNP), HERC2:rs12913832:A>G, that is located in an enhancer element of the Oculocutaneous albinism type II (OCA2) gene. In addition to HERC2:rs12913832:A>G, five SNPs (OCA2:rs1800407:G>A, SLC24A4:rs12896399:G>A, TYR:rs1393350:G>A, SLC45A2:rs16891982:C>G and IRF4:rs12203592:C>T) were previously found to be the best predictors of human eye colour. Together, these six SNPs are termed the IrisPlex SNPs. Nevertheless, many eye colours cannot be explained by only considering the IrisPlex SNPs.
In the search for other eye colour factors, we sequenced the entire coding region of OCA2 and 500 kb upstream of the OCA2 start codon in 68 unrelated Scandinavian and Italian individuals and compared the sequences with quantitative eye colours of all individuals. A total of 47 samples originated from subjects with unexpected eye colours based on the genotype of HERC2:rs129138332:A>G. The samples came from (1) subjects with dark eye colours and the genotype HERC2:rs12913832:GG, and (2) subjects with light eye colours and the genotype HERC2:rs12913832:GA.
We found the nonsynonymous variants OCA2:rs1800407:G>A, OCA2:rs74653330:C>T, and OCA2:rs121918166:A>G to act as penetrance modifiers of HERC2:rs129138332:A>G, and resulted in light eye colours of individuals with HERC2:rs129138332:GA. Interestingly, OCA2:rs74653330:C>T and OCA2:rs121918166:A>G were only found in individuals of Scandinavian descent. Our study also showed that the key to explain the unexpected dark eye colours in HERC2:rs12913832:GG individuals, may be found outside the HERC2-OCA2 region. Preliminary results showed that other genes, including SLC24A4, are important for the development of dark eye colours.

474. SINGLE/DOUBLE EYELIDS ASSOCIATED GENES IN EASTERN

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Predicting donor's Externally Visible Characteristics (EVCs) based upon DNA intelligence, Forensic DNA Phenotyping (FDP) provides leading information for police investigation when reference DNA is absent. In a primary investigation, we have evaluated the relationship between 4 candidate SNPs and single/double eyelids [1]. In this study, candidate genes massively parallel sequencing (MPS) association study was conducted to investigate SNPs and/or haplotypes related to single/double eyelids to provide potential facial morphology associated genetic markers used in forensic individual identification of Asian population. PCR amplification, agarose gel electrophoresis, gel extraction, purification, library construction and sequencing (with Miseq® FGx, Illumina) of fragments on 19 candidate genes were performed one by one within gDNA extracted from 24 Chinese Han heathy volunteers with typical single/double eyelids. Data was tested with HWE and LD. 33 SNPs on BMP4, SPRY2 and HBEGF genes indicated genotype and/or allele frequency differences between single/double eyelids. 7 haplotypes on the three genes implied significant difference of frequency between single/double eyelids. 7 Tag SNPs in 5 haplotypes were tested through Haploview 4.2.

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475. THE CONFIRMATION OF GENETIC VARIANTS ASSOCIATED WITH THE SUBJECTIVE RESPONSE AFTER ALCOHOL CONSUMPTION

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Association studies have identified a number of genetic variants associated with the subjective responses to alcohol. The most extensively studied ones were nine SNP among the gene of *ADH1B*, *ADH1C*, *ALDH2*, *CYP2E1*5B*, *CHRNA3*, *GABRA2*, *OPRM1*, *HYKK* and *SLC6A4*. However, it still needs further confirmation. In the present study, fourteen subjects were administered 0.8 g/kg of alcohol. Their subjective response was assessed by filling out SHAS and carrying out pursuit rotor task at 2h, 4h and 8h after alcohol consumption. Besides, these nine SNPs of each subject were genotyped with multiplex SNaPshot method. Repeated measures analysis of variance indicate that the subjects with *SLC6A4 A/C* show significantly stronger subjective feelings of alcohol intoxication than that of subjects with C/C ($P=0.042<0.05$). The genetic variants of *SLC6A4* is associated with the level of subjective response to alcohol in the preliminary trial.

476. THE IMPACT OF DIFFERENT CARRIER MATERIALS ON THE STABILITY OF DNA METHYLATION LEVELS FOR AGE

PREDICTION AFTER LONG TERM STORAGE OF HUMAN BLOOD STAINS

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It has been shown that methylation analysis using DNA from fresh blood samples can be used for age prediction with a reasonable level of accuracy, demonstrating its potential application also in criminal casework on forensic stain samples. Further steps, to assess the stability of DNA methylation should be covered in order to ensure reproducibility of results. Both carrier material and long term storage could potentially induce DNA methylation alterations and subsequently, their potential effect on these epigenetic markers should be explored.
In our study, we have analyzed blood stains from two donors stored at room temperature for up to 30 years on the following carrier materials: paper, wood, leather, wool, wallpaper, carpet, blue denim, and nylon stockings. The stability of DNA extracted from these stains was assessed by STR analysis. Using bisulfite pyrosequencing we analyzed the methylation pattern at six CpG loci in the genes of *ASPA*, *ITGA2B*, *PDE4C* and *ELOVL2* that are already known to be suitable age-predictive markers. The stability of the DNA methylation levels was assessed per carrier material and storage time. Age estimations were performed using two previously reported age prediction models (Weidner et al., Genome Biol. 2014, 15:R24; Zbieć-Piekarska et al., 2015, FSI Genet. 14:161). Differences between epigenetic and chronological age were assessed and discussed. The results of our ongoing study will provide valuable information on the effect of both storage periods as well as carrier substrates on the outcome of methylation-based age prediction in the forensic context.

477. THE USEFULNESS OF THE AGE PREDICTION METHOD IN FORENSIC DNA SAMPLES

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Currently, the age estimation method using DNA methylation has been developed such as pyrosequencing method and SNaPshot by single base extension. In this study, we tested two different age prediction methods which methods are more applicable to forensic field samples. We selected 5 CpG markers(ELOVL2, C1orf132, TRIM59, KLF14, FHL2) which were known to the most significantly correlated age estimation markers with blood, and 3 CpG semen markers(cg06304190, cg12837463, cg06979108)[1,2]. In 5 blood markers were tested by the Pyrosequencing system, 3 semen markers by the methylation SNaPshot method. Concordance test and limited of detection test of the two methods were performed. We collected DNA from blood and semen from the same person, and tested three times. As the result, the lowest detection limit of blood was 4ng/ul in pyrosequencing, semen was 1.25ng/ul in SNaPshot, the error range was ± 4.6 yrs in blood, ± 4.0 yrs in semen. Result of the concordance test, it was almost same age between the estimated age from the blood and semen. We also applied 5 blood CpG markers to the epithelial DNA(from the buccal swab). There were no significant differences between blood DNA and buccal swab DNA in age prediction. The detectable DNA concentration of the Pyrosequencing method was more higher than the SNaPshot, but it was more simple and handy. In conclusion, it is considered two age estimation methods could be useful in forensic science field in the future.

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478. UNRAVELING THE COMPLEX GENETICS OF HUMAN HEAD HAIR FEATURES TO FACILITATE THEIR PREDICTION ACCURACY IN FORENSICS

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Hair shape, hair loss and hair greying are highly heritable and variable traits and thus comprise attractive targets for DNA-based prediction in forensics. High variation of hair shape and loss is observed at the intercontinental level and different genomic regions have been shown to play role in determination of these characteristics in Europeans and Asians. The complexity is further enhanced by the possible overlap between genetic variants linked to various head hair features. Nonetheless, the percentage of the total variation in the aforementioned hair characteristics explained by genes remains low and prediction accuracy is limited.

To further our understanding of the genetics of hair shape, loss and greying, we validated an extended set of 207 candidate SNPs in a population of ~700 individuals from Poland investigated as a part of a research project NEXT. The project assumes developing DNA predictive system for human appearance involving massively parallel sequencing provided by the Ion AmpliSeq™ technology and Ion S5™ system and complex prediction modeling. To address inter-population differences and facilitate prediction at the global level, genotypic data were additionally gathered for ~100 phenotypically characterized individuals from East Asia and relevant genotypes for Africa retrieved from 1000 Genomes database. Our study reveals genetic link between hair shape and loss and assess the practical value of predictive analysis of these externally visible characteristics in various human populations.

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479. VICTIM IDENTIFICATION BY GENETIC ANALYSIS IN A HUMAN DISASTER

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After the each human disaster, Identification of victims is the most subject for relatives. Genetic methods are very important strategies for burned and degraded human remains. A dreadful disaster occurred in an industries facility near the Tehran city. In this event, killed 21 persons. Corpses of the victims were divided into 115 pieces. Some of them could be identified with their medical case history and their external signs. But a great number of bodies were burned and divided to different separated parts without special identification sign. The body parts, DNA extracted by RGDE[1] methods and profiled by 16Plex Identifiler and Yfiler Kits. Inheritance is high power technique. The results analyzed by NoorGis national software and Familias software[2]. The blood samples derived from the relatives and DNA extracted and profiled by the same manner and compared with victims profiles and all of them identified with the highest amount of paternity index (PI). From two identified victims, a paternity relationship was demonstrated. The results show that the DNA finger printing is the best and rapid method for unknown victims Identification in mass disaster investigations [3].

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480. EXTENSIVE POPULATION STRUCTURE IN THE Y-CHROMOSOME LANDSCAPE OF SAUDI ARABIA REVEALED BY ANALYSIS OF 27 Y-STRS

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Saudi Arabia is a largely desert country, with a population historically organized into patrilineal descent groups, or tribes. To date, little has been done to characterize its population structure, and particularly to assess patterns of diversity in the male-specific region of the Y chromosome. We have used the 27-STR YFiler Plus kit to generate haplotypes in ~600 unrelated indigenous Saudi males, classified into five broad geographical regions (north, south, central, east and west). We also used Y-STR haplotypes to predict Y-SNP haplogroups. Haplogroup J1 dominates the whole country with an overall frequency of 70%, but is significantly more common (>80%) in the geographical center (north, central and southern

regions). By contrast, the frequency in eastern and western regions is only ~50%. Mean heterozygosity across all Y-STR loci is 0.528 for the sample as a whole, and ranges from 0.413 (north) to 0.630 (east). Patterns based on predicted haplogroups are also reflected in STR genetic diversity values (Rst) and in the distribution of haplotypes within STR networks. Of the 600 males recruited for the study, 100 were Saudis studying in the UK. Despite ensuring equal representation of the five geographical regions in both UK- and Saudi-recruited cohorts, their distributions of predicted haplogroups differ significantly ($p<0.001$).Overall, our study reveals a remarkably high degree of structuring of paternal lineages by geographical region, and in addition likely social structuring affecting haplotype distributions on the basis of sampling strategy. These findings should be accommodated in forensic interpretation of Y-STR profiles in Saudi Arabia.

481. DRAMATICALLY INCREASING THROUGHPUT FOR DNA Y-SCREENING OF SEXUAL ASSAULT EVIDENCE BY 96-WELL PLATE PROCESSING

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Backlogs of untested sexual assault evidence kits have become a priority for U.S. law enforcement agencies in recent years. Y-Screening with DNA is growing as the fastest solution for detecting male DNA (usually from sperm) in sexual assault evidence kits and estimating the probability of obtaining a suspect profile from the collected evidence. Advancements in direct-amplification chemistries and robotics are continually being made, though much continues to be relatively labor intense and remain in single tube form. In order to make any significant reduction in time, evidence samples need to be processed in 'plate format' (usually 96 well), reducing many cumbersome manual manipulations. This however, is usually avoided by laboratories because of the risks such as sample-switching, cross-contamination, and sample tracking.
Sorenson Forensics has evaluated several approaches to overcome this set of risks and the obstacles of Y-screeening processes. This novel high-throughput approach was developed and tested as an improved streamlined Y-screening process that utilizes the newly developed Promega Casework Direct kit, Promega SlicPrep plate devices, robotic processing, and a new instrument specifically designed to assist with precisely building 96 well evidence plates. With the utilization of these components, laboratories could potentially see an estimated three to four-fold increase in DNA Y-Screening throughput.

482. A COMPROMISING POSITION FOR A VARIANT: A NEW VARIANT OF D1S1656 THAT INVADES ITS NEIGHBORS AND CAN LEAD TO MISSINTERPRETATION

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Short tandem repeats (STRs) are the markers of choice for purposes of human forensic identification

because of their considerable degree of polymorphism. This variability may occasionally become a challenge for the analyst when a new variant invades the allele distribution range of the neighboring *locus*. We present here a novel variant at *locus* D1S1656 showing a molecular length of 211.32 bp corresponding to 21.3 repeating units. This variant is superimposed on the D12S391 *locus* when the Global Filer™ (Thermo Fisher Scientific Inc., USA) is used, invading the shortest allele range and being assigned as Off-ladder (OL). In contrast, typing with PowerPlex® Fusion (Promega Corp., Madison, USA) overlaps the D2S441 *locus*, generating an allele that is recognized as 8. In both cases the invasion was observed as a tri-allele pattern. The results were confirmed by DNA re-extraction and typing with the two independent commercial megaplexes described above. The variant was detected during analysis of a reference sample throughout a paternity exclusion case. This is the first time this variant has been described and reported to the NIST STR Base. Since D1S1656 is included in widely-used multiplex kits from several vendors - Biotype (Dresden, Germany) Qiagen (Venlo, The Netherlands), Promega (Madison, USA) and Thermo Fisher (Foster City, CA, USA) - it would then be recommendable that other forensic labs be aware of this new micro-variant in dealing with similar interpretation challenges and that the kit producers take this fact into account in designing new multiplex kits.

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ISFG 2017 SEOUL

General Information

ABOUT KOREA

The Korean Peninsula is located in North-East Asia. It is bordered by the Amnok River (Yalu River) to the northwest, separating Korea from China, and the Duman River (Tumen River) to the northeast which separates Korea from both China and Russia. Because of its unique geographical location, Korea is a very valuable piece of land and an international hub of Asia. The country itself is flanked by the Yellow Sea to its west and the East Sea to the east. There are several notable islands that surround the peninsula including Jejudo, Ulleungdo and Dokdo.

Nowadays, Incheon Airport continues to rank first in airport service evaluation, being the representative of our country's airports. Up to now, 88 airlines have settled into Incheon Airport, reaching out to 195 cities of 54 countries. It is handling 3,986 flights per a week, 24 hours. With averagely 13 minutes of exiting and 18 minutes of entering the border checkpoint, it maintains the world's highest level of speed. The fastest transfer can be done within 45 minutes, being the most competitive among the neighbor countries. There are approximately 40,000 members of staff providing various kind of service for passengers. With theme facilities, events, exhibitions for entertainment, passengers hold a high degree of satisfaction towards Incheon airport after use.

National flag, Taegeukgi

National flower, Mugunghwa

WEATHER




Korea's climate is regarded as a continental climate from a temperate standpoint and a monsoonal climate from a precipitation standpoint. The climate of Korea is characterized by four distinct seasons: spring, summer, fall, and winter.

FOOD


Order food in a Korean restaurant and you'll soon find your table filled with dishes. The basics of Korean cuisine are bap (rice), guk (soup), and kimchi. Korean people's love for bap, or rice, is unique. However varied or delicious the side dishes may be, a Korean meal is almost always centered around rice.
Try bulgogi or bibimbap, a favorite among tourists, as their taste is easy on the first time eaters of Korean cuisine. Rice cake and traditional tea are also strongly recommended as they have a delicate flavor unlike more typically spicy and pungent Korean food.

CURRENCY

The currency in Korea is the Korean Won (KRW).
Foreign currency and travelers' check can be exchanged at bank and hotels.
Most shops accept all major credit cards.
Notes: KRW1,000 / KRW5,000 / KRW10,000 / KRW50,000
Coins: KRW10 / KRW50 / KRW100 / KRW500

BUSINESS HOURS

Banks
- Weekdays: 09:00 – 16:00
- Weekends and National Holidays: Closed

Post Offices
- Weekdays: 09:00 – 18:00
- Saturdays: 09:00 – 13:00
- Sundays & National Holidays: Closed

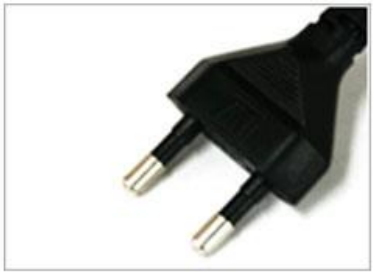
Department Stores
- 10:30 – 20:00

* Typically one day a month (usually a Monday) department stores are closed to the public. However, closings will vary according to each store.

USEFUL PHONE NUMBER

Number	Information	Language Service
1330	**24 hour Korea Travel Hotline** Various tourism information inquiries and complaints	English, Chinese, Japanese
120	**Seoul Dasan Call Center** Various inquiries regarding Seoul	English, Chinese, Japanese, Vietnamese, Mongolian
119	**National Rescue Service** Request for help with fire, natural disasters and emergency situation	
112	**Korea National Police Agency**	
1345	**Korea Immigration Service**	20 Languages
1588-5644	**BBB Korea (Before Babel Bridge)** 24-hour interpretation service	19 Languages

Electricity and Voltage



The standard voltage in Korea is 220 volts. The outlet has two round holes and is the same type used in France, Germany, Austria, Greece, Turkey, and many other countries. If you do not have a multi-voltage travel adapter, you can borrow one from your hotel's front desk. If you want to buy one in Korea, you can do so at a duty-free shop, convenience shop at Incheon International Airport.

ISFG 2017 SEOUL

PROGRAM AT A GLANCE

Monday, August 28, 2017

09:00 - 18:00 Workshop 1 (Room 301A)
09:00 - 18:00 Workshop 2 (Room 307)
09:00 - 13:00 Workshop 3 (Room 301B)
09:00 - 13:00 Workshop 4 (Room 300)
11:00 - 11:30 Coffee Break
13:00 - 14:00 Lunch(Coex Mall, B1)
14:00 - 18:00 Workshop 5 (Room 300)
14:00 - 18:00 Workshop 6 (Room 301B)
16:00 - 16:30 Coffee Break

Tuesday, August 29, 2017

09:00 - 18:00 Workshop 7 (Room 403)
09:00 - 18:00 Workshop 8 (Room 300)
09:00 - 18:00 Workshop 9 (Room 301A)
09:00 - 13:00 Workshop 10 (Room 301B)
11:00 - 11:30 Coffee Break
13:00 - 14:00 Lunch (Coex Mall, B1)
14:00 - 18:00 Workshop 11 (Room 301B)
16:00 - 16:30 Coffee Break
18:30 - 19:30 ISFG Scientific Prize Lecture
19:30 - 20:00 Opening Ceremony (Auditorium)
20:00 - 21:30 Welcome Reception (Grand Ballroom 103+104)

Wednesday, August 30, 2017

09:00 - 13:00 Session 1 (Auditorium)
10:30 - 11:30 Coffee Break
10:30 - 11:30 Poster Session (Grand Ballroom 101+102)
13:00 - 14:30 Lunch (Coex Mall, B1)
* For the non-registrant
13:00 - 14:30 Lunch Seminar (Auditorium, Grand Ballroom 103+104)
*For the pre-registrant
14:30 - 18:15 Session 2 (Auditorium)
16:00 - 17:00 Coffee Break
16:00 - 17:00 Poster Session (Grand Ballroom 101+102)
18:30 - 19:30 Working Group Meeting (See p18)

Thursday, August 31, 2017

09:00 - 13:00 Session 3 (Auditorium)
10:30 - 11:30 Coffee Break
10:30 - 11:30 Poster Session (Grand Ballroom 101+102)
13:00 - 14:30 Lunch (Coex Mall, B1)
* For the non-registrant
13:00 - 14:30 Lunch Seminar (Auditorium, Grand Ballroom 103+104)
*For the pre-registrant
14:30 - 18:15 Session 4 (Auditorium)
16:00 - 17:00 Coffee Break
16:00 - 17:00 Poster Session (Grand Ballroom 101+102)
18:00 - 19:30 General Assembly of the ISFG (Auditorium, ISFG members only)

Friday, September 1, 2017

09:00 - 13:00 Session 5 (Auditorium)
10:30 - 11:30 Coffee Break
10:30 - 11:30 Poster Session (Grand Ballroom 101+102)
13:00 - 14:30 Lunch (Coex Mall, B1)
* For the non-registrant
13:00 - 14:30 Lunch Seminar (Auditorium, Grand Ballroom 103+104)
*For the pre-registrant
14:30 - 17:00 Session 6 (Auditorium)
16:00 - 16:15 Coffee Break
18:00 - 18:30 Transportation
18:30 - 22:00 Gala Dinner & After Party (Grand Walkerhill Seoul)
*Only the participants who purchased ticket

Saturday, September 2, 2017

09:00 - 11:15 Session 7 (Auditorium)
10:00 - 10:15 Coffee Break
11:15 - 12:00 Closing Ceremony (Auditorium)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