Theme 1: Population genetics of polymorphisms of forensic interest

Genetic variation for 20 STRs loci in a Northeast Colombian population (Department of Santander)

A. Castillo[1], A. Gil[1], A. Pico[1], C. Vargas[1], I. Yurrebaso[2], O. Garcia[2]

[1]Universidad Industrial de Santander, Bucaramanga, Santander, Colombia.

[2]Forensic Science Unit, Basque Country Police (FSU), Erandio, Bizkaia, Spain

Before a new marker system can be introduced into forensic casework, a population database for the relevant population must be established for statistical evaluation of the evidence. Therefore, this report presents allele frequency data in a Northeast Colombian population sample.

DNA samples from 500 unrelated individuals from different provinces of Santander department were amplified, typed and their allele frequencies were determined. Results demonstrate the assumption of independence within and between the loci analyzed.

We analyzed 20 autosomal STRs markers included in the commercial kit PowerPlex® 16 and GenePrint Fluorescent STR Multiplex GenePrint -F13A01, FESFPS, F13B, LPL- (Multiplex FFFL) and PowerPlex® System CS7. The amplified products were separated and detected using an ABI 310 sequencer and the allele assignment was performed by Genescan software v. 2.1 and Genemapper ID v. 3.2.

Statistical evaluations were performed using different computer programs. Analyses included the possible divergence from Hardy-Weinberg expectations and other parameters of forensic importance: observed and expected heterozygosities, mean exclusion chance (MEC), polymorphic information content (PIC), discrimination power (DP) and the possible associations between loci.

Therefore, a Northeast Colombian population database can be used in identity testing to estimate the frequency of a multiple PCR-based locus DNA profile.

Genetic polymorphism of 30 InDel markers for forensic use in Bangladeshi population

S. Akhteruzzaman[1], S.A. Das[1], I Hosen[1], A Ferdous[2]

[1]Department of Biochemistry & Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh.

[2]National Forensic DNA Profiling Laboratory, Dhaka Medical College, Dhaka-1000, Bangladesh.

Insertion-deletion polymorphisms (InDels or DIPs) represent a large portion of all polymorphisms in human genome. They are basically length polymorphisms created by insertion or deletion of one or more nucleotides, combining the common features of both SNPs and STRs. Their low mutation rate, smaller amplicon size and multiplexing capability made them suitable for use in forensic and parentage testing. In this study, we genotyped a set of Bangladeshi population sample (n=132) using 30 InDel markers included in Investigator DIPplex PCR amplification kit (QIAGEN, Germany). Allele frequency, observed and expected heterozygsity (H), polymorphism information content (PIC), probability of match (PM), power of discrimination (PD), typical paternity index (TPI) and power of exclusion were calculated for these loci. Hardy-Weinberg equilibrium tests demonstrated no significant deviation from expected values (P > 0.00167, after Bonferroni correction for multiple testing). The random probability of match was 2.87 ´ 10-12 and cumulative power of exclusion was 0.99470. The high levels of power of discrimination (0.999999875) makes it well suited for identification of individuals. But relatively low range of TPI (0.791 – 1.179) and PE (0.0685 – 0.263) limits its use fullness in paternity and kinship investigations. The studied 30 InDel loci however offers a good supplementary tool for resolving challenging kinship studies and an efficient alternative to SNP typing in the studied population.

Population genetics data on 15 STR markers of Ceara state, Northeast region of Brazil

D. Andrade [1], S. Rodriguez[2], F. Vasconcelos[2], D. Campos[1], A. Souza [2], R. Bezerra [3], M. Melo[1], R. Carvalho[1], M. Floringo[2], J.J. Builes[4], A. Gaviria[5].

[1]Central Public Health Laboratory (LACEN), Ceará, Brazil

[2] Department of Nutrition, Ceará State University, Brazil

[3]Department of Patology, Ceará Federal University, Brazil
[4]Laboratorio GENES Ltda, Medellin, Colombia
[5]Laboratorio de Genetica Molecular de Cruz Roja Ecuatoriana y Cruz Vital S.A. Quito, Ecuador.

Ceara population is 8,452,235 people, of whom 32% is Whites, 4.65% Blacks, 1.25 % (Asians) yellow, 61.88% Pardos and 0.23% Natives (Indigenous). The aim of this study was to complement the population data on this region of Brazil.

For this study the blood samples of 590 non-related individuals were used. DNA was extracted using the Chelex-100 method. The amplification was carried out using the Power Plex 16 kit (Promega Corporation). This kit allows the detection of the following loci: D3S1358, THO1, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA. The detection by capillary electrophoresis using an ABI3130 Genetic Analyzer

Based on allele distribution, the forensic parameters for each locus were calculed. The population sample under study did not show any deviation from Hardy – Weinberg or Linkage disequilibrium.

Fifteen autosomal microsatellite data from Ecuador

A. Gaviria[1,2], A.K. Zambrano[1,2], G. Morejon[1,2], V. Aguirre[1,2], M. Vela[1,2], J.J. Builes[3], G. Burgos[1,2]

[1] Laboratorio de Genética Molecular de Cruz Vital S.A. Quito, Ecuador.

[2] Cruz Roja Ecuatoriana, Quito, Ecuador.

[3] Laboratorio GENES Ltda, Medellin, Colombia.

Allele frequencies for 15 autosomal STRs (D5S818, D7S820, THO1, TPOX, VWA, CSF1P0, D16S539, D13S317, D3S1358, D8S1179, FGA, PENTA D, PENTA E, D21S11, D18S51) were estimated from a sample of 1100 unrelated individuals living in Ecuador.

The DNA was extracted from whole blood following the FTA method (Whatman). The PCR amplification was performed using Power-Plex 16 system, according to manufacturer's instructions (Promega). The PCR products were separated in an ABI PRISM 3100 Genetic Analyzer (Applied Bioystems) and the typing analysis method used was the Genemapper version 3.1.2 software.

Quality control: Proficiency testing by the GEP-ISFG WG (http://www.usc.es/gep-isfh) and SLAGF

Statistical analysis and results: Heterozigosity values (expected) were calculated according to Nei. Several forensic and population

parameters were estimated by using the Cervus 2.0 and Powerstats software.

Other remarks: No deviation from Hardy–Weinberg equilibrium was detected at any locus. In addition, distribution of allelic frequencies shown in the present study was compared to other published data from Ecuador with no statistical significant differences in any system.

The Evaluation of 23 STR Multiplexing System Used in Forensic Testing

Jian-xin[1]; Shuang-lin[1]; Hong[1]; Wei-xia[2]; Chao[1]; Wei-an[3]; Jie[3]; Xiao-hui[1]

[1]Guangzhou Forensic Science Institute, Guangdong Province Key Laboratory of Forensic Genetics, Guangzhou

[2]Institute of Forensic Medicine, Southern Medical University, Guangzhou

[3]AGCU ScienTch Incorporation, Wuxi

The new building 23 Multiplexing system EX23 is a polymerase chain reaction-based amplification kit that include 22 autosomal STR loci CSF1PO, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D2S441, D3S1358, D5S818, D6S1043, D7S820, D8S1179, FGA, PentaD, PentaE, TH01, TPOX,vWA and a Y STR loci DYS391. As the system is designed for further practical application, series of regorous testing is developed to ensure the reliability for genotyping purpose.

We tested the systems by observe its sensitivity, accuracy, balance and its genotyping ability when the materials are mixtures, contact stains and degraded samples. A total of 196 unrelated Chinese individuals in Guangdong province were typed to evaluate the system and examine concordance for 15 loci which targeted in widely-used commercial kit sinofiler. All samples were extracted by MAXWELL, then separated on ABI3130XL genetic analyzer and analyzed by GeneMapper ID 3.2 software.

The genotyping results can be well obtained from0.005ng DNA, which is accurate and balance. The mixtures, degraded samples and contact stains were also successfully typed. The systems' total discrimination power is 0.999999999, cumulative probability of paternity exclusion for triplet cases is 0.999999997.

The new building 23 Multiplexing system get satisfactory testing results and show high polymorphism in Guangdong Han population, thus, it can confidently be used for forensic and human identification test.

A multiplex of eight pentameric-repeat STRs novel to forensic profiling

C. Phillips[1], M. Gelabert-Besada[1]*, L. Fernandez-Formoso[1]*, L. Prieto[2], M.V. Lareu[1].

[1] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain.

[2] University Institute of Research Police Sciences (IUICP), DNA Laboratory, Comisaría General de Policía Científica, Madrid, Spain.

** Contributed equally to the study*

Pentameric-repeat STRs have the advantage of showing very reduced stutter product peaks compared to their tetrameric-repeat STR counterparts. This characteristic provides clearer patterns in mixed DNA profiles when minor component alleles can coincide with the higher stutter peak signals of tetrameric repeat STRs and thus hinder interpretation. To provide a simple mixture interpretation multiplex we have developed, de novo, a 10-plex assay of eight pentameric STRs new to forensic applications plus DYS391 and the Y-Indel marker recently incorporated in the GlobalFiler STR kit[†].

We describe the peak patterns obtained, construction of allelic ladders, stutter ratios and the levels of polymorphism observed in the HGDP-CEPH global population panel for the eight pentameric STR components of the multiplex.

*[†] **Acknowledgement:** The authors are indebted to Lisa Calandro, Life Technologies, for providing locus details to allow independent primer designs for the Y-Indel marker of GlobalFiler.*

New and supplementary forensic STRs: A review

C. Phillips[1], L. Fernandez-Formoso[1], M. Gelabert-Besada[1], M. Garcia-Magariñas[2], J. Amigo[3], S. Kind[1], M.V. Lareu[1]

[1] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain.

[2] Department of Mathematics, University of A Coruña, Spain.

[3] Grupo de Medicina Xenómica, University of Santiago de Compostela.

The STR kits available to forensic practitioners have changed markedly in the last two years so that multiplexed sets of core STRs anticipating CODIS expansion now include Life Technology GlobalFiler typing 22 STRs, and Promega Powerplex-21 plus Powerplex Fusion typing 20 and 23 STRs respectively. In addition, the Qiagen HDplex STR kit brings nine novel STRs and Promega CS-7 adds a further five STRs to supplement a core set of 24 STRs (including D6S1043 of Life Technology SinoFiler and Promega Powerplex-21 kits).

We have validated eighteen STRs with the 944-sample HGDP-CEPH global population panel to compliment a previous study of the same panel for twenty core forensic markers (C. Phillips et al., 2011). The STRs studied comprised core loci: SE33, Pentas D/E and D6S1043 plus 14 supplementary loci from Qiagen HDplex and Promega CS7. We report global allele frequencies, rare and off-ladder alleles observed and forensic informativeness metrics. Interestingly, the high discrimination power of D6S1043 is not confined to East Asians but is strong in all populations studied.

Since the combination of Promega Fusion and Qiagen HDplex provides 32 unique STRs from just two kits, we examined the power of this set of STRs to improve relationship testing statistics, particularly applied to challenging cases such as distant pairwise comparisons in deficient pedigrees.

Lastly, we outline upgrades to the pop.STR forensic STR allele frequency browser to now encompass comprehensive population data for 24 core, 14 supplementary and 14 specialist forensic STRs.

C. Phillips et al., Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STRs using the CEPH human genome diversity panel, Forensic Sci. Int. Genet. 5 (2011) 155–169.

Allele frequencies for 35 autosomal STR loci in a Norwegian population sample and two immigrant populations from Africa and Asia

B.M Dupuy[1], D. Kling[1,2], M. Stenersen[1]

[1]Norwegian Institute of Public Health, Department of Family Genetics, Oslo, Norway

[2]Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Aas, Norway

In order to update our frequency databases for all autosomal STR

loci currently used in the daily routine, DNA-profiles from unrelated Norwegians (N=11000) as well as two immigrant populations from Africa (N=800) and Asia (N=730) have been analysed. This is important to obtained unbiased likelihood ratios (LR) when using Familias in paternity testing and, more importantly, more distant family relationships where frequencies of rare alleles can be crucial to the final conclusion. All samples were pair wisely compared to discard any unknowingly related individuals.

Allele frequencies for 35 autosomal loci (23 loci included in PP16, PP18D or ESX17 from Promega 10 additional loci in HD-plex from Qiagen, together with APOAI1, DS11554, and D17S906) will be presented in adequate tables. Statistical parameters of forensic interest (Power of Discrimination, Power of Exclusion, Matching Probability, Estimation of kinship, etc.) will also be presented and some concordance data between kits will be discussed.

Characterization of sequence variations in the D21S11 locus in Danes, Somalis and Greenlanders by next generation sequencing

E. Rockenbauer[1], S. Hansen[2], M.R. Andersen[1], E.F. Heerup[1], C. Børsting[1], S.L. Fordyce[1], N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[2]Centre of Forensic Genetics, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

Next generation sequencing made it possible to study sequence variations of STR loci in an inexpensive way. We recently developed a robust 454 FLX pyrosequencing method for this purpose. Complex STR loci with several sub-repeat sections are most informative in term of sequence diversity. D21S11 is a highly polymorphic core STR locus with a complex sequence structure. We have recently identified D21S11 variants with equal lengths but different sub-repeat compositions. Our results also indicated that the sub-repeat patterns of the D21S11 alleles in Caucasians and Africans were different. The aim of this study was to investigate the sequence variation and allele frequencies of D21S11 alleles in three geographically distant populations, Danes, Somalis and Greenlanders. A total of 127 unrelated individuals from Danish paternity and immigration cases belonging to the three ethnic groups were sequenced on a GS Junior System (Roche Diagnostics). The sequences were screened, sorted and aligned. In total, 36 allele variants with 14 different lengths were identified among 256 alleles. The highest degree of variation was found in Somalis and the lowest in Greenlanders. Some of the variants were present in two, or in all three groups. However, 27 variants were only found in one of the groups.

Mutational Patterns of Autosomal Tetranucleotide and Pentanucleotide Microsatellites in Human

Y.C. Tai[1], C. Liu[2], H.Y. Li[3], E.P. Huang[1], P.M. Qiu[1], L. Chen[1], W.Y. Tang[3], F. Zhang[3], H.J. Wang[1*]

[1]Department of Basic Medical Sciences, South Medical University, Guangzhou, China;

[2]Institute of Criminal Technology of Guangzhou, Guangzhou, China;

[3] Forensic Science Centre of Guangdong Provincial Public Security Department, Guangzhou, China

[*]Correspondence to: Huijun Wang, Department of Basic Medical Sciences, Southern Medical University, 1838 North Guangzhou Road, 510515. E-mail: hjwang@fimmu.com

Introduction: Microsatellite mutation mechanism and its associated factors are not completely understood.

Aims: It is of great forensic importance to study the underlying mechanism.

Materials and methods: In the present study, we studied 240 mutation events within 156,505 parent/child allelic transmissions in 6356 families.

Results and discussion: Mutation rates that increase exponentially over the size of the longest run of perfect repeats (LRPR) were found in 12 loci, in which most determination coefficients are above 0.80. Our mutation data analysis showed a bias toward expansion (113 expansions vs. 82 contractions, $Z = 0.031$) and longer alleles are prone to contraction. Regression analysis of expansion mutation rate (EMR) and contraction mutation rate (CMR) showed that an exponential curve fits better than a linear one does. We found EMR and CMR curve crossed nearby the standardized allele size of 0.7. Loci with $[CTTT]_n$ motif have higher mutation rate than those with $[TCAT]_n$ motif, possibly implying the effect of non-B DNA conformations. The observation of an excess of paternal mutations (male-to-female ratio of 4.7) supports a male bias of mutation in microsatellites. Effect of paternal age on mutation was demonstrated by two methods in the present study, whereas no effect was found for maternal age. Our data is supportive for the replication slippage hypothesis but deviates in part from the stepwise mutation model (SMM), indicating that microsatellite mutation is a complex process.

Key words: microsatellites; STR; mutation; replication slippage; evolution; the SMM

Identification of new primer binding site mutations at TH01 and D13S317 loci and determination of the point mutation-STR haplotypes

H. Pang[1], J.H. Zhang[2], F.R. Li[1,3], J.F. Xuan[1], J.X. Xing[1], M. Ding[1], B.W. Wang[1]

[1]School of Forensic Medicine, China Medical University, Shenyang, Liaoning, P.R. China

[2]Shenyang Center for Disease Control and Prevention (CDC), Shenyang, Liaoning, P.R. China

[3]Department of Forensic Medicine, Baotou Medical College, Baotou, Neimenggu, P.R. China

Several commercial multiplex PCR kits for the amplification of short tandem repeat (STR) loci have been extensively applied in forensic genetics. Consequently, large numbers of samples were genotyped and less discordant genotypes were also observed. We found two novel alleles dropout at the two STR loci TH01 and D13S317 during paternity testing by use of the AmpFlSTR® Identifiler® PCR Amplification Kit. The lost alleles reappeared when alternative PCR primer pairs were used. Sequencing analysis revealed a G-to-A substitution at position 82 bases downstream of the last TCAT motif of the repeat region on TH01 locus (GenBank Accession: D00269) and a G-to-T substitution at position 90 bases upstream of the first TATC motif of the repeat region on D13S317 locus (GenBank Accession: G09017). Allele frequency of the two point mutations was subsequently investigated in a Chinese population by sequence specific primer PCR technique (SSP-PCR), but no other sample was detected in both loci. In addition, to determine the point mutation-STR haplotypes on the two loci, the DNA samples with identified mutations were first amplified to type the point mutations by SSP-PCR, subsequently, the obtained haploid PCR products with different point mutations and STR repeat numbers were directly sequenced so that the strategy overcomes overlap peaks appearance generated from different STR alleles and may characterize

accurately genotypes. Thus, our findings not only complemented useful information for DNA database and forensic identification and but also built up an effective strategy for haplotype's typing with primer binding site mutation's STR.

The genetic diversity and quantitative ethnicity analysis of the Uyghur population, Forensic Science International: Genetics

X. Pan[1], Ji Qian[1], P.F. Qin[1], W.F. Jin[1], S. Xu[1], L. Jin[1]

[1]MOE Key Lab of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China

Population-specific genetic markers could help us to identify ethnic origins of individuals. The Uyghur population, an ethnic minority settled in Xinjiang, China, presents a typical admixture of East Asian and European anthropometric traits. The previous studies in our laboratory have shown that the Uyghur have received almost equal gene flow from both East Asian and European -related ancestry. The variation of genetic contribution from both ancestral populations at individual level is very minor.

In this study, we exploited genome-wide high-density single nucleotide polymorphisms (SNPs) to develop a set of more reliable genetic markers, which would have some extended applications to avoid the limitation of Short tandem repeats (STRs) on paternity testing. We also developed a set of ancestral informative markers (AIMs) by population genetic and statistical approach based on Uygur population-specific SNPs, i.e. 45 AIMs comprised of highly population-differentiated SNPs were identified based on the Eurasian populations from the International HapMap Project Phase 3 and 1000 Genomes Project. Consequently, we developed an experimental pipeline to identify whether an individual originates from Uyghur, which have some special advantage compared with the traditional forensics marker set that is currently used. We genotyped 133 individuals from the Uyghur, Kazakh and Kyrgyz populations using Affymetrix SNP Array 6.0. Principal component analysis and STRUCUTRE analysis showed that the 45 AIMs have sufficient power to distinguish the Uyghur individuals from either European populations or East Asian populations. Then we genotyped the 45 AIMs in 794 Uyghur individuals and further assessed the performance of the AIMs.

Genetic characterization of a population from the Department of Cauca - Colombia with 15 STRs markers

J.J. Builes[1,2], C.H. Afanador[3], A Manrique[1,2], D.P. Aguirre[1], C.M. Velilla[1], J.F. Castro[1,2], L Mendoza[1,4], M.L.J Bravo[1]

[1] Laboratorio GENES Ltda, Medellín, Colombia.

[2] Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

[3] Universidad de Pamplona, Pamplona, Colombia.

[4] Universidad Pontificia Bolivariana, Medellín, Colombia

The molecular markers STR are a widely used tool in forensic genetics and biological anthropology. This study established allele frequencies and some parameters of forensic interest from a sample of 172 unrelated individuals from the Department of Cauca in Colombia using the PowerPlex® 16 BIO System kit (Promega).

Samples of 172 unrelated individuals from the Department of Cauca in Colombia were taken. The DNA was isolated by salting-out and Chelex 100. For PCR was used the PowerPlex® 16 BIO System kit (Promega). The amplified products were separated by electrophoresis in 6% polyacrylamide gels and were visualized with a 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 (one per locus) yields an actual significance of 0.0033 (Weir, 1966). The forensic parameters were calculated with PowerStats software (Promega CO). Genes Ltda participates annually in interlaboratory quality controls with the Spanish and Portuguese Speaking Working Group of ISFG (GHEP-ISFG) and with the Colombian Group Human Identification and Forensic Genetics (GCIHyGF).

All markers analyzed showed more than 61% heterozygosity. Penta E and Penta D were the only systems that are not 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 0.9999, 0.9999 and > 0.9999, respectively. The parameters of forensic interest had values suitable for routine use in forensic genetics.

Analysis of 15 autosomal STR Loci in the Population of the State of Acre, Brazilian Amazon

L. Birolo[1,2], A. Amorim[1,2], C. Alves[2], A.G.G. Mesquita[3], L. Gusmão[2,4]

[1]Department of Biology, Faculty of Sciences of the University of Porto (FCUP), Porto, Portugal;

[2]Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal;

[3]Center of Biological and Natural Sciences, Federal University of Acre (UFAC), Rio Branco, AC, Brazil;

[4]DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro - RJ, Brazil

Acre was the last state of Brazil to be inhabited by non-indigenous individuals. The "Acrianos" are mostly descendant of Northeasterners and of the original indigenous inhabitants. The aim of this research was to construct a database specific to the population of Acre and to compare Acre to other populations.

The genetic characterization of each of the five geopolitical regions of the state was performed by determining the frequencies of 15 autosomal Short Tandem Repeats (STRs) included in the amplification kit AmpFlSTR® Identifiler® Plus (Applied Biosystems), for a total sample of 503 non-indigenous individuals.

The FST values did not reveal significant differences amongst regions in the state. For all loci, the population does not show significant deviation from the Hardy-Weinberg equilibrium. At the national level, Nei's genetic distances between our sample, Brazil in general and two indigenous groups, show a lower distance between Acre´s and the indigenous populations than between the Brazilian and the indigenous populations, while comparison of Acre with other populations (from Brazil, several African and European countries, Mexico, and countries bordering to Brazil) confirms that Acre is well integrated in the Brazilian context. Moreover, in South America only Bolivia and Peru show relatively large distances for all considered populations including Acre. When comparing this database with others currently used in forensic testing in Acre (from general Brazil and the Amapá state), significant values were found for 5 out of the common 12 loci considered, indicating that these databases may not be suitable for the application to Acre´s population.

Using STR, MiniSTR and SNP markers to solve complex cases of kinship analysis

A. Ibarra[1], M. Martinez[1], A. Freire-Aradas[2], M. Fondevila[2], A. Carracedo[2], L. Porras[3], L. Gusmão [4,5]

[1] IdentiGEN - Genetic Identification Laboratory and Research Group of Genetic Identification, School of Natural and Exact Sciences (FCEN), University of Antioquia, Medellin, Colombia

[2] Institute of Forensic Sciences, Genomic Medicine Group, University of Santiago de Compostela, Galicia, Spain

[3] Technological University of Pereira, Colombia

[4] IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

[5] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro - RJ, Brazil

In complex kinship investigation, miniSTRs and SNPs have been frequently used in order to increase the likelihood ratio (LR), when the results obtained for the most commonly used STR multiplexes were not informative enough. In this work, we describe the results obtained when using a battery of 23 STRs, 3 miniSTRs and 52 SNPs (SNP*for*ID 52plex identification panel; Sanchez et al., 2006; Electrophoresis 27:1713–1724) to investigate three complex paternity cases where the father was not available, and one paternity case with bone samples, from which no results could be obtained for STRs (including the 3 miniSTRs, D10S1248, D14S1434 and D22S1045). These four cases were selected from the routine cases at the laboratory IdentiGEN at the University of Antioquia, based on the low LR obtained after typing the STR and miniSTRs (W<99.99%, p=0.5, which is considered in Colombia as non-conclusive; http://www.secretariasenado.gov.co/senado/basedoc/ley/2001/ley_0721_2001.html)

The LRs were calculated using the allele frequencies observed in Colombian population samples: for the STRs and miniSTRs we have used the database from routine paternity cases at the IdentiGEN laboratory at the University of Antioquia, comprising unrelated individuals living in Colombia; for the 52 SNPs included in the SNP*for*ID 52plex, we have used previously published data from the West-Central Andean Region (Ibarra et al., 2013; Int J Leg Med., in press, DOI: 10.1007/s00414-013-0858-z).

In all cases, the additional information provided by the SNP*for*ID 52plex identification panel (Sanchez et al., 2006; Electrophoresis 27:1713–1724) was enough to achieve conclusive results.

Evaluation of the PowerPlex Fusion system in a sample from East Timor

L. Souto[1,2], F. Tavares[1], H. Moreira[1] and F.Corte-Real[2]

[1] Department of Biology, University of Aveiro, Aveiro, Portugal

[2] Medicine Faculty, University of Coimbra; CENCIFOR – Forensic Science Center, Coimbra, Portugal

East Timor (Democratic Republic of Timor-Leste) is a country located in Southeast Asia that consists of 13 districts, including the eastern half of the island of Timor, the Ataúro island, Jaco and Oecussi (an enclave on the northwestern side of the island).

In this study, we determined allele frequencies and forensic parameters for 24 STR autosomal loci included in the PowerPlex® Fusion System (Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179, D12S391, D19S433, FGA and D22S1045).

Autosomal STR data were collected from saliva samples of 100 individuals from East Timor. The amplification of the 24 STR autosomal was performed using PowerPlex® Fusion System (Promega Corporation) and the amplified products were analyzed on a 3500 Genetic Analyzer using GeneMapper® ID-X 1.2 Software (Applied Biosystems). The most polymorphic loci were Penta E, D21S11 and D18S51. All the analyzed loci meet Hardy-Weinberg equilibrium after Bonferroni correction. Data from East Timor population are contextualized in the frame of Southeast area/Pacific region. In our samples, we found "off-ladder" alleles (ex: D2S441, Penta E and FGA locus) confirmed by reamplification, amplification with others kits and sequenced when justified.

This project is funded by the FCT Project: PTDC/CS-ANT/108558/2008 TIMORPOP GENéTICA DE TIMOR-LESTE, Operational Programme Thematic Factors of Competitiveness (COMPETE) and European Community Fund FEDER.

Population data for Central Portugal Population with NGM amplification kit

A.M. Bento[1, 2], A. Semo [1], V. Lopes [1, 2,] V. Bogas[1, 2], P. Brito[1, 2], A. Serra[1, 2] L. Andrade[1, 2], L. Souto [2,4], F. Corte-Real, F.[2, 3], M.J. Anjos[1, 2]

[1] Forensics Genetic Service, Central Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal

[2] Cencifor, Forensic Science Centre, Portugal

[3] National Institute of Legal Medicine and Forensic Sciences I.P., Portugal

[4] Department of Biology, University of Aveiro, Portugal.

The AmpFlSTR NGM Amplification kit in one of the recent amplification kits that includes the new European Standard Set (ESS) miniSTR loci (D1S1656, D2S441, D12S391, D10S1248 and D22S1045). As this new markers are already in use in most forensic laboratories, it is necessary to have accurate population data for those markers.

The purpose of this work was to establish allele frequencies and population statistic parameters for Central Portugal population using NGM amplification kit.

DNA was extracted from buccal swabs by chelex 100 method from 150 healthy unrelated individuals from Central Portugal, involved in paternity testing after consent. PCR amplifications were performed using the AmpFlSTR NGM Amplification kit according to manufacturer's instructions in a GeneAmp 9700 PCR System. Electrophoresis was carried out on ABI PRISM™ 3130 Genetic Analyser and data analysis and allele calling was done using GeneMapper V3.2 analysis software. Relevant forensic parameters and allele frequencies were determined using PowerStat v1.2 software package and Hardy-Weinberg equilibrium was assessed using Arlequin software v 3.5.

No significant deviations from Hardy-Weinberg equilibrium were observed, except for D2S441 (p<0.05). Allele frequencies obtained for the five loci included in the new ESS set are similar to those from other European populations. The inclusion of these markers in STR kits provided a powerful tool for forensic genetics, as they are highly informative.

Patterns of maternal and paternal ancestry in Griqua populations

P.G. Ristow[1], K.R. Heynes[1], L.S. Jurado Medina[2], C.M. Bravi[2], G. Bailliet[2,] M.E. D'Amato[1]

[1] Forensic DNA Laboratoty, University of the Western Cape, Republic of South Africa.

[2] Instituto Multidisciplinario de Biología Celular (IMBICE), La Plata, Argentina.

The Griquas are an admixed group who, during the 1800s, were forcefully relocated from the Western Cape to nomansland in East Griqualand. They are believed to carry genetic similarities with Khoi-khoi who were the original inhabitants of the Cape. The aim of this study was to analyze the genetic diversity of the Griqua population in Kokstad and Vredendal. Ninety four Griqua, male and female, samples were collected from Kokstad (63) and Vredendal (31) within the Republic of South Africa.

The Control Region of the mitochondrial genome was amplified and sequenced. Haplogroups of each sample were identified using HaploGrep. Thirty five male samples were typed using an exploratory Amplified Product Length Polymorphism (APLP) method, Y-Filer and UWC-10-plex STR system.

The maternally inherited haplogroups showed different ancestral

compositions with a higher percentage of Khoi-khoi ancestry (87% L0d) in the Vredendal group. The Kokstad group (48% L0d) displayed a heterogeneous haplogroup composition. The majority of the Kokstad population belonged to Y-chromosome haplogroup R (58%) whereas haplogroup Q (57%) was present in the highest quantities in Vredendal. While a pattern of paternal European and African haplogroups are predominant in the Kokstad group, the Vredendal group displayed Asian and African ancestry. The gene diversities of the STR systems also showed differences with the Y-Filer having a lower gene diversity (0.654) than the 10-plex (0.837). Currently a large effort is in place to sample more individuals from six Griqua settlements in South Africa.

This work was funded by the Department of Science and Technology of the Republic of South Africa (DST) and the Ministry of Science, Technology and Innovation of Argentina (MINCyT)

Genetic variation in a Japanese population using the multiplex 24 STRs analysis system

M. Hashiyada[1], K. Usui[1], Y. Hayashizaki[1], N. Hosoya[1], Y. Igari[1], J. Sakai[1], M Funayama[1]

[1]Division of Forensic Medicine, Department of Public health and Forensic Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan.

For a successful identification, more STR regions have been required with the increasing use of the DNA database. In this report, we performed multiplex 24 STRs analysis in 407 unrelated Japanese and built a Japanese database.

Genomic DNA extracted from blood was amplified by the PowerPlexR Fusion System (Promega) which contains 24 STRs, that is 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, as well as Amelogenin and DYS391 for gender determination. Electrophoresis and allele typing were carried on Applied Biosystems 3500 Genetic Analyzer and GeneMapper ID-X ver.1.2 software (Applied Biosystems).

Forensic statistics parameters, such as the observed and expected heterozygosity, matching probability (MP), polymorphism information content (PIC) and probability values of the Hardy–Weinberg equilibrium were calculated by PopwerStats spreadsheet (Promega) and Arlequin ver3.5. The result revealed the most highly polymorphic locus was Penta E (MP:0.017 and PIC:0.902), and TPOX showed the lowest value (0.169 and 0.602). The combined matching probability value for 22 loci excluding the Amelogenin and DYS391 was 4.13 x 10-26. Furthermore, no significant deviation from Hardy-Weinberg Equilibrium was detected.

We concluded that these 24 STRs analysis offer high effectiveness for forensic and genetic application.

Mutation or exclusion: when siblings are not siblings

N. Cerri[1], V. Cortellini[1], A. Verzeletti[1], De Ferrari F[1].

[1]Department of Forensic Medicine, University of Brescia, Brescia, Italy

Deficient paternity cases, where the DNA profile of the alleged father is not available, are not unusual in forensic practice. Considerably larger sets of genetic markers have to be examined than in standard casework and the statistical evaluation of the DNA evidence is more difficult. Such a case can also be burdened with danger of false inclusion.

The following case of disputed paternity has recently come at our attention. The claimant pretended to be the natural daughter of a long-time deceased man, who had three legitimate children, two sons and one daughter.

Initially, genetic profiles were obtained from three subjects: the claimant, the alleged father's daughter and one alleged father's son. The results for a 20 STRs loci profile revealed only one exclusion between the claimant and the siblings. Also the analysis of X chromosome polymorphisms revealed one exclusion. Only by typing for 20 STRs loci also the other son the combined results revealed five exclusions.

In cases when the request is to verify the relationship between two half-siblings of different gender in the absence of data from parents, when sexual chromosomes polymorphisms are not useful, the only possible approach is to increase the number of analyzed autosomal STRs and include in the analysis the most number of parents as possible.

Polymorphisms analysis and evaluation of five non-CODIS STR loci in a population sample of immigrants living in Northern Italy

V. Cortellini[1], N. Cerri[1], A. Verzeletti[1], F. De Ferrari[1]

[1]Department of Forensic Medicine, University of Brescia, Brescia, Italy

Northern Italy ranks among the highest proportion of immigrants from non-EU countries, amounting about 14% of the local population. Data regarding the allelic frequencies for STRs in these populations would be highly welcomed to be used in forensic genetics for identification and paternity testing.

Moreover ENFSI recently (European Network of Forensic Science Institutes) introduced in forensic DNA typing five new STRs loci (D10S1248, D22S1045, D2S441, D1S1656, D12S391) not included in CODIS. It is necessary to obtain data regarding allele frequencies for these five loci in different population in order to verify the usefulness of these new markers, allowing their use in forensic cases. The five new autosomal STRs loci represent markers able to improve the discriminatory power of forensic analysis and to enhance genotyping success when analyzing highly degraded DNA by amplifying fragments well below current average amplicon sizes.

Until now the five new STRs loci lack extensive population studies, therefore it is necessary to obtain data regarding allele frequencies in different populations in order to verify the usefulness of these new markers, allowing their use in forensic cases.

In this study two groups of individuals of two of the main ethnic groups living in Northern Italy, the Albanians and the Indo-Pakistanis, were typed using the commercial kit AmpFlSTR®NGMÔ (Applied Biosystems, Foster City, CA, USA); allelic frequencies and forensic parameters were calculated.

PowerPlex® Fusion kit: a 23plex autosomal STR kit for human identity testing

A. Verzeletti[1], V. Cortellini[1], N. Cerri[1], A. Bosetti[2], F. De Ferrari[1]

[1]Department of Forensic Medicine, University of Brescia, Brescia, Italy

[2]Promega Italia srl, Milano, Italy

In case of paternity or maternity investigations with short tandem repeat (STR) analysis, deficient cases, missing persons, or mutations are encountered and sometimes common STRs cannot provide good results. Thus, it is recommended that additional STRs are used to complement conventional analysis for more reliable forensic information.

We analyzed variation of 23 STRs contained in the new PowerPlex® Fusion kit (Promega) in 52 unrelated individuals involved in paternity testing casework to contribute to create an Italian database. In

our laboratory, this new kit is used as a screening tool to solve deficient cases as fatherless paternity test, and to help in paternity investigations with only one genetic incompatibility after the use of routine fifteen loci.

Allele frequencies and forensic parameters were used to evaluate suitability and robustness of the new kit for forensic genetic analysis as well as in concordance studies with other kit (Identifiler and NGM - Applied Biosystem).

Through this new kit a picture of allelic frequencies for 23 STRs loci in a population sample from Northern Italy was provided.

6 Mini-STR loci (D1S1677, D2S441, D4S2364, D10S1248, D14S1434, D22S1045) Gene Frequency in Turkey

G. Filoglu[1], E. Sipahi[1], H. Altuncul[1], G.Rayimoglu[1], O. Bulbul[1]

[1] Institute of Forensic Sciences, Istanbul University,Turkey

In Forensic Sciences, DNA analysis is required for identifications, paternity test, understanding the relationships between people, and establishment of a connection between the creme scene and the quilty person. In order to adapt six mini STR loci for Turkish Criminal Laboratories. The alelle frequency of those loci in Turkish Population must be determined. The aim of the study is to determinate the alelle frequencies of D10S1248, D14S1434, D22S1045, D4S2364, D2S441, D1S1677 mini STR loci in Turkish population and to became a valuable tool for Turkish Criminal Laboratories. First of all, DNA was isolated from blood samples, which were taken from 200 volunteer subjects. After that, 6 STR loci were amplified by two different multiplex PCR reaction. Primer set was combined as FAM-HEX-TET based on the method "New Mini STR loci D10S1248, D14S1434, D22S1045, D4S2364, D2S441, D1S1677 validation and optimization on blood samples" (2011) published by Unsal T et al. For this dye set, DS-34 Matrix standart and TAMRA-350 size standart were applied on ABI 310 genetic analyser. PCR condition was the same with the article which was published by Coble M.D. (2005). Allele Frequencies were calculated with Powerstats V.1.2 (Promega Cooperation) population genetics Excel workbook. Hardy Winberg Equlibrium was tested for each Loci with Arlequin 3.1 population istatistic program. Finally, In order to compare our data with other populations we calculate Z score for every allele.

Expanding the allele base for STR analysis using sequence polymorphisms

P. Ivanov[1], S. Leonov[1], E. Zemskova[1], T. Timoshenko[1]

[1]Federal Center for Forensic Medical Expertise of Ministry of Health of Russian Federation, Moscow, Russia

Using the Abbott PLEX-ID PCR/mass spectrometry-based STR assay covering a total of 13 CODIS loci, we typed 300 blood samples from unrelated Russian individuals all had full STR profiles generated with the conventional ABI Identifier Plus/CE system.

The key elements of Abbott PLEX-ID PCR/ESI-TOF-MS platform are the measurment of PCR product masses via electrospray ionization-time of flight-mass spectrometry, determination of product base compositions from their masses, and the association of the product base compositions to the alleles of particular locus. It enables to determine when an allele has a SNP within the amplified region because the polymorphism changes the mass of the PCR product.

The typing results were consistent with conventional typing results at all loci. In addition, sequence-based polymorphisms relative to the reference alleles were seen in 10 of the 13 loci tested. From 2 variant alleles in FGA up to 15 in D5S818 were observed. At the 8 loci at least one representative heterozygous individual was found for which the

locus is called homozygous by conventional typing. The observed frequency of occurrence of each variant relative to reference alleles was calculated along with discriminating power (DP) and power of exclusion (PE).

The results demonstrate potential advantages of the sequence-based polymorphisms assay in resolution of samples when limited number of loci can be STR-typed.

Genetic analysis of g.7260-7291 region of ANKRD1 in Japanese population

S. Nakamura[1], C. Murakami[1], M. Maeda[1], W. Irie[1], C. Sasaki[1], T. Watanabe[1], M. Ohishi[1], K. Kurihara[1]

[1]Department of Legal Medicine, Kitasato University School of Medicine, Sagamihara, Japan

Short tandem repeat (STR) polymorphisms are powerful tools for human identification, paternity analysis and genetic mapping. A new dinucleotide repeat marker located on g.7260-7291 region in intron 3 of ankyrin repeat domain 1 (ANKRD1) was analyzed, in order to obtain genetic data for Japanese population.

DNA was extracted using QuickGene-800 (FUJIFILM) from 248 Japanese healthy individuals living in Kanagawa prefecture with informed consent. Primers covered g.7260-7291 region of ANKRD1 were designed and PCR amplification with PrimeSTAR GXL DNA polymerase (TAKARA) was performed using GeneAmp PCR system 9700 (Applied Biosystems). PCR products were sequenced by direct sequencing using BigDye Terminator v.3.1 Cycle sequencing Kit (Applied Biosystems) and electrophoresed using ABI 3130 Genetic Analyzer (Applied Biosystems). The resulting sequence data were compared with the reference sequence available on the NCBI database (NG023227.1). This study was approved by the Kitasato University Medical Ethics Committee (B03-17),

In the present study, a total of 26 genotypes were detected in 248 individuals and these were considered to be controlled by 9 alleles composed by [TA]4CA[TA]2, [TA]10, [TA]12, [TA]13, [TA]14, [TA]15, [TA]12[TTTA]2, [TA]17 and [TA]18. These alleles were designated as 7a, 10, 12, 13, 14, 15, 16a, 17 and 18, and allele frequencies were calculated as 0.450, 0.026, 0.008, 0.252, 0.046, 0.010, 0.183, 0.014 and 0.010, respectively. The distribution of genotypes fitted the Hardy-Weinberg equilibrium. The power of discrimination, heterozygosity and polymorphism information contents were 0.863, 0.697 and 0.743, respectively. These results suggested that this new dinucleotide repeat marker was useful for individual identification in forensic caseworks.

Sib test using the PowerPlex 21 System

T. Tamura[1,2], M. Osawa[2], A. Nakanishi[2], K. Miyashita[2], T. Nakamura[1]

[1]Forensic Biology Unit, Scientific Crime Laboratory, Kanagawa Prefectural Police, Yokohama, Japan

[2]Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Japan

The latest multiplex PCR system used in forensic science contains over 20 autosomal STR loci. The conventional system employing the AmpFLSTR Identifiler kit, which is consisted of 15 STR loci, is short of the significant evaluation in sib test. In the present study, performance of the PowerPlex 21 System composed of 20 STR loci in pairwise test was compared to that of the Identifiler System. Based on the Japanese allele frequencies, full-sibling and unrelated pairs were constructed on simulation. The combined sibship indices (CSI) were calculated for each pairs by multiplying likelihood ratio values

according to the product rule. The 15 loci analysis to unrelated pairs revealed incomplete exclusion when the significant CSI value was over 1,000. In the 20 STR loci, none of unrelated pairs were misjudged and 89.7% of the sib ones were properly judged as full-sibling when the threshold was 500. But, the CSI distributions in the two groups were still overlapped in part. The additional STR loci give rise to problem of linkage in inherence. The PowerPlex System contains two sets of closely located STR loci. CSF1PO and D5S818 are 24 cM apart and D12S391 and vWA are 12 cM apart on the same chromosome. Linkage disequilibrium between these STR loci was calculated using the Arlequin algorism for Japanese parent-child pairs, full-siblings, and unrelated persons. The result indicated that the loci were apparently linked in the relationships. However, it was hard to choose one from several proposed procedures for correction.

Maternal DNA mutation at D21S11 in a paternity testing involving a child with Down Syndrome

U. Ricci[1], I. Carboni[1], S. Iozzi[1], A. Gozzini[1], F. Torricelli[1]

[1]AOU Careggi, SOD Diagnostica Genetica, Florence, Italy

Complex kinship analyses in which the alleged father is not available can be resolved using a large panel of STRs markers. Hereby we show a paternity test in which a deceased alleged father was suspected to be the biological father of a child affected of Down syndrome. The mother and two half-brothers of the child were available for DNA testing. A panel of 37 autosomal STRs markers and 16 Y-STRs including commercially and manual systems were applied. Evaluation by using Familias® program confirmed the paternity, but at D21S11 the child showed three different alleles with just one identical at the mother. In order to further investigate the parental origin of the extra chromosome 21 and the meiotic/mitotic origin a panel of seven STRs marker mapped in the chromosome 21 normally used for prenatal diagnostics via QF-PCR were also analyzed in the mother and in the child. The used 21 STR markers were mapped along the whole chromosome from position 21q21.3 to 21q21.1 The results show that the observed inconsistency was due to a maternal mutation. This study clearly shows that there was single step mutation in the locus D21S11 in the germ cell of the mother. The predisposition to this meiotic error observed in this report is compatible with the widely reported in the literature for the origin of trisomy 21. In fact just 5% of Down syndrome occur during spermatogenesis, while errors in meiosis that lead to trisomy 21 are overwhelmingly of maternal origin.

Forensic and population genetic analyses of eighteen non-CODIS miniSTR loci in the Korean population

H.J. Jin[1], K.C. Kim[2], C. E. Yoon[3], W. Kim[2*]

[1]Department of Nanobiomedical Science, Dankook University, Cheonan 330-714, Korea

[2]Departement of Biological Sciences, Dankook University, Cheonan 330-714, Korea

[3]Biowithus Life Science Institute, Seoul 133-120, Korea

We analyzed variation of eighteen miniSTR loci in 411 randomly chosen individuals from Korea to increase the probability that a degraded sample can be typed, as well as for providing an expanded and reliable population database. As part of the present study, six multiplex PCR systems were developed (multiplex I: D1S1677, D2S441 and D4S2364; multiplex II: D10S1248, D14S1434 and D22S1045; multiplex III: D12S391, D16S3253 and D20S161; multiplex IV: D3S4529, D8S1115 and D18S853; multiplex V: D6S1017, D11S4463 and D17S1301; multiplex VI: D5S2500, D9S1122 and D21S1437). Allele frequencies and forensic parameters were calculated to evaluate suitability and robustness of these non-CODIS miniSTR systems. No significant deviation from Hardy-Weinberg equilibrium expectations were observed, except for the D4S2364, D5S2500 and D20S161 loci. A multidimensional scaling (MDS) plot based on allele frequencies of the six miniSTR loci (D1S1677, D2S441, D4S2364, D10S1248, D14S1434 and D22S1045) showed that Koreans appeared to have the most genetic affinity with Chinese and Japanese than to other Eurasian populations compared here. The combined probability of match (PM) calculated from the 18 miniSTR loci was 2.902 × 10-17, indicating a high degree of polymorphism. Thus, the 18 miniSTR loci can be suitable for recovering useful information in analyzing degraded forensic casework samples and adding supplementary genetic information for a variety of analyses involving closely related individuals where there is a need for additional genetic information.

Genetic polymorphisms of 20 STR loci in Chinese Han population in Tianjin, North China

B.Wang[1,2], J.Z.Kuang[2], Y.Ma[2], G.M.Wang[2], W.Zhu[2], Y.P.Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

[2]Institute of Forensic Science of Tianjin Public Security Bureau, Tianjin 300384, Tianjin, China

The PowerPlex® 21 System PCR Amplification Kit was a new PCR Amplification Kit developed for forensic laboratories, but there was a lack of data about this kit in Chinese population in Tianjin, North China. This kit contained 20 STR loci, D3S1358, D1S1656, D6S1043, D13S317, Penta E, D16S539, D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, D8S1179, D12S391, D19S433 and FGA. In order to evaluate this kit and to get basic population data for its use in forensic practice in Chinese Han population, 360 unrelated Chinese Han individuals from Tianjin were typed using the Kit. Allele frequencies of the 20 STR loci and further population forensic genetic parameters were obtained. The observed genotype frequencies and expected genotype frequencies were evaluated by x2 test. No significant deviation from the Hardy–Weinberg equilibrium was observed in our population sample for the 20 STR loci. The population data in the present study can be used for routine forensic practice in Tianjin, North China. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK16B01), the National Natural Science Foundation of China (81273349).

Polymorphism of vWFII in Chinese Han population and its application to forensic science

X. Wang[1]

[1]School of Criminal Investigation, Southwest University of Political Science and Law, Chongqing, China

To get preliminary genotype and allele frequency distributions of vWF intron 40 nt31/1890–1991 (vWFII) locus in Chinese Han population and to validate it for forensic application.

The vWFII locus was amplified on DNA samples. The PCR products were analysed by PAG vertical electrophoresis followed by silver staining. Furthermore, the nested PCR was carried out to improve the quality of PCR and PAGE with very tiny amount of templates.

Nine alleles were found at vWFII locus, spanning from 103bp to 135bp. A total of 29 genotypes were observed in 282 individuals. The results of test for Hardy-Weinberg equilibrium showed that the genotype distributions observed were correspondent with the expected (P 0.05). The heterozygosity was 0.81. The polymorphism information content (PIC) was 0.7942. The chance of exclusion was 0.5813 and

the discriminating power was 0.929. Using nested PCR we can type correctly from tiny template DNA of 1µl blood extraction, while by direct PCR the amplification products could not be observed clearly on PAG.

The vWFII locus may be a very useful genetic marker for both paternity test and personal identification of casework in forensic science and for the purpose of population genetics. The method of nested-PCR could be useful for the STR-PCR typing when the specimen is in tiny quantity.

Theme 2: Forensic DNA Databases

The Victorian Missing Persons DNA Database – Two Interesting Case Studies

A. Stock[1], L. Benton[1], M. SPiden[1], and D. Hartman[1,2]

[1] *Victorian Institute of Forensic Medicine, 57-83 Kavanagh St, Southbank VIC 3006, Australia.*

[2] *Department of Forensic Medicine, Monash University, Australia.*

Investigations into persons missing and presumed dead require the collation of information regarding the missing person, ante-mortem (AM) records collected before the person's disappearance, with information obtained from the post-mortem (PM) examination of unknown deceased persons. In most missing persons cases, the ante-mortem information includes personal information as well as any dental and medical records; with some also including fingerprint information.

In Victoria, this information is captured by Victoria Police using Plass Data. Whilst Plass Data can be used to record vital DNA profiling information, it lacks the ability to conduct searches of the DNA data for direct or kinship matches between missing persons and unidentified deceased.

In 2010, the Victorian Institute of Forensic Medicine (VIFM) in collaboration with Victoria Police established the Victorian Missing Persons DNA Database (VMPDD) – capable of conducting kinship and direct searches using both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) profiling data. Here we describe two interesting case studies:

- The first highlights the need to conduct at least two types of DNA analysis – such as Short Tandem Repeat (STR), Y-STR, or mtDNA analysis – to confirm a match; and
- The second describes the successful reconciliation of an unknown deceased with a missing person case – that would have otherwise gone unsolved.

These cases highlight the importance of such a database to identify missing persons.

The National Criminal Investigation DNA Database (NCIDD) - Bringing More Science to Australia's National DNA Capability.

Carmen Eckhoff[1]

[1]*National Biometric and Child Protection Services, CrimTrac Agency, GPO Box 1573, Canberra, ACT, 2601; carmen.eckhoff@crimtrac.gov.au*

In 2001 CrimTrac established the National Criminal Investigation DNA Database (NCIDD). The NCIDD is a web-based application designed to view potential links between DNA records at the jurisdictional and inter-jurisdictional level. The NCIDD was initially established to assist law enforcement agencies across Australia to compare DNA profiles from crime scenes with DNA profiles from convicted offenders to either identify or eliminate them as potential suspects in other crimes.

At 1 August 2013, the NCIDD held approximately 758,000 DNA profile records. The majority (95%) of the profiles held in the NCIDD were created using a ten (10) loci multiplex kit.

To better serve law enforcement agencies, CrimTrac is considering expanding the capability of NCIDD to be more than a direct match engine thus capitalising on available capabilities in DNA investigations.

CrimTrac will explore options and implement a solution that will enable our law enforcement partners to benefit through the analysis of DNA for criminal purposes, DVI and reconciliation of long term missing person with unknown human remains.

The benefits that accrue from implementing a national DNA Investigative Capability include:

- Increase in the effective and timely DNA identification of disaster victims and long term missing persons.
- Increase in the resolution of unsolved major crime cases.
- Increase in the number of jurisdictions using the CrimTrac DNA platform as the sole DNA capability.
- Alignment of DNA business drivers, strategies, goals and objectives between the Jurisdictions and CrimTrac will be improved; and this will facilitate efficient and effective policing throughout Australia.

The impact and advantages of expanding the U.S. core autosomal STR markers

C.R. Hill[1], M.D. Coble[1], M.C. Kline[1], J.M. Butler[1], P.M. Vallone[1]

[1]*National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, USA*

The original set of 13 Combined DNA Index System (CODIS) autosomal short tandem repeat (STR) loci are currently required for upload of DNA profiles to the U.S. national DNA database. As the number of profiles continues to increase each year, the likelihood of adventitious matches becomes greater. Expanding the core loci from 13 to 20 (including DYS391) is critical to reduce the potential of these types of matches occurring within the database, to increase international compatibility for data sharing (e.g. D1S1656, D2S441, D10S1248, D12S391, D22S1045), and to increase discrimination power in missing persons and complex kinship cases.

Commercial companies have recently released next-generation STR multiplex kits (PowerPlex Fusion and GlobalFiler Express) that enable

complete coverage of all of these additional loci for a total of 24 loci in each kit. These kits have been extensively tested at NIST using 3130xl and 3500 Genetic Analyzers, allowing the probability of identity calculations to be made with different sets of loci and population statistics determined with a standard set of unrelated U.S. population samples from 4 groups (n=1036). These loci have been characterized to determine the impact that this additional information will have on database searches. A variety of forensic genetic parameters have been analyzed with the NIST data set including allele frequencies, heterozygosities, peak height ratios, mutation rates, and stutter percentages.

The efficiency of Y-chromosome markers in forensic trace analysis and their inclusion in the Austrian national DNA database

F. Neuhuber[1], E. Klausriegler[1], G. Kreindl[1], W. Zahrer[1], B. Dunkelmann[1], J. Camper-Kiesslich[1]

[1] Institute of Legal Medicine, University of Salzburg, Austria

The fields of application of Y-chromosome DNA markers (Y-STRs) in forensics are to be found first in the establishment of kinship via continuous male genealogical lines, and second in the analysis of biological traces in which a mixture of male and female trace material is to be expected. The latter is frequently the case especially in sexual offences. In Austria a start was made in 2012 to include Y-STRs in the National DNA Database, with the primary objective to identify serial sexual offences.

Forensically, the analysis of sperm secretion traces from sexual assault cases is comparatively simple. However, in such offences there is often no ejaculation or only contact traces are transferred. Since these contact traces are usually mixed with traces of the victim, it is often impossible to generate an autosomal, and hence database-suitable, male DNA profile due to an excess of female DNA. Y-STR profiles, on the other hand, can easily be generated even if there is still an approximately 1,000-fold excess of female DNA.

Since 2010 our laboratory has been subjecting traces from all sexual offences not only to autosomal analysis but also to Y-STR analysis. We have found that from a considerable number of analyzed traces it is possible to generate a Y-chromosome DNA profile even if all the prior sperm tests are negative and no sign of any male component is found in amelogenin. In 38 of a total of 239 sexual offences a perpetrator trace was identified solely using Y-STR analysis.

Based on these findings, the Austrian National DNA Database was expanded to include Y-STRs. Initially, it involves traces only, and no reference samples. The first results of these efforts are presented.

An integrated STR database of the Philippine population: the genetic diversity of Filipinos at 23 aSTR and 23 Y-STR DNA markers

J.J.R. Rodriguez[1, 2], J. Salvador[1], A. Agmata[1], L.C. Carandang[1], D.L. Apaga[1], A.R. Espigar[1], G. Calacal[1], R. Laude[2], and M.C. De Ungria[1]

[1]DNA Analysis Laboratory, Natural Sciences Research Institute, College of Science, University of the Philippines, Diliman, Quezon City, Philippines

[2]Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines, Los Baños, Laguna, Philippines

The probative value of DNA evidence depends on the number of markers analyzed and their levels of variability in the relevant population. A highly discriminating DNA database of the Philippines was established from allele frequencies of 23 autosomal short tandem repeat (aSTR) and 23 Y-chromosomal STR (Y-STR) loci among Filipinos.

The availability of this dataset facilitates the combined use of aSTR and Y-STR markers in order to individuate and identify the source of male DNA in cases which require complex analysis, e.g., mixture deconvolution of sexual assault evidence. A joint match probability should be determined to identify the offender with correction for weak association between aSTR genotypes and Y-haplotypes.

DNA from 167 unrelated Filipino males originating from the three major island groups that comprise the Philippine archipelago, Luzon, Visayas and Mindanao, were analyzed at 23 aSTRs (D1S1656, TPOX, D2S441, D2S1338, D3S1358, FGA, D5S818, CSF1PO, D6S1043, D7S820, D8S1179, D10S1248 TH01, vWA, D12S391, D13S317, Penta E, D16S539, D18S51, D19S433, D21S11, Penta D, and D22S1045) and 23 Y-STRs (DYS19, DYS385 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS481, DYS533, DYS549, DYS570, DYS576, DYS635, DYS643, and Y-GATA-H4).

The most informative aSTR marker is Penta E, with Polymorphism Information Content (PIC) of 0.91. Combined Matching Probability (CMP = 1 in 1.29664×10^{27}), and combined power of discrimination (CPD = 0.99999999999999999999999993) of the aSTR data were also computed. With 167 unique Y-STR profiles detected, no haplotype is shared among individuals.

Italian National Forensic DNA Database in an European Perspective

V. Marchese[1], N.Cerri[2], L.Caenazzo[1]

[1] Dept. of Molecular Medicine, University of Padua, 35121 Padova , Italy

[2] Dept. of Forensic Medicine, University of Brescia, 25123 Brescia, Italy

Italian law n. 85/2009 ratifies the Prüm Treaty which is an agreement of seven EU Member States regarding cooperation in justice and home affairs and establishing the highest possible standard of cooperation of exchange of information, particularly in combating terrorism, cross-border crime and illegal migration. This law regulates the institution of the Italian national forensic DNA database and introduces modification articles of the Code of Penal Procedure relative to the technical assessments that can affect personal freedom involved in biological sampling and collection of DNA profiles. Even though DNA databases are a great value for investigation and society, it may be troublesome to solve the balance between public interest in crime control and individual rights such as personal freedom, dignity and privacy.

The purpose is to consider the distinctive features of the genetic data. Particularly, their immutability, predictability on the future health conditions and sharing within the so called "biological group" makes really difficult to find a proper management of the genetic data.

The crucial issue to be taken into consideration is the ethical one, which concerns the processing of this sensitive private information. Our interest is to compare the solutions provided by international standards and by sentences, both European and Italian, with articles 12 and 13 of the law n. 85, that regulate security measures and storage of DNA profiles and biological samples. The critical analysis of the Italian regulation testifies that a fair balance between individual rights and social security is extremely hard to fulfill.

A study of the first DNA databank of sexual assault evidences in Brazil

S. T. G. Ferreira[1], K. A. A. Paula[1], F. A. S. Maia[1], A. Trindade-Filho[1], C. R. Mendes[1], H. I. Pak[1], G. C. Daltonl[1], L. C. Arantes[1], A. S. Svidzinski[1], M. R. Amaral[1], S. A. Diniz[1], A. V. Moraes[1]

[1]*Instituto de Pesquisa de DNA Forense - IPDNA - Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil*

DNA evidence from sexual assaults and DNA databanks are very important tools for crime investigations and identification of perpetrators. In Brazil, DNA testing for criminal investigations was performed for the first time by the Instituto de Pesquisa de DNA Forense, IPDNA, Polícia Civil do Distrito Federal in Brasília, the capital of Brazil, in 1995. At that time, since in Brazil there were no legislation regarding DNA databank of suspects or condemned, the IPDNA created the first DNA databank of sexual assaults evidences in Brazil to help investigations. The aim of this study is to show the experience of the IPDNA in solving crimes using a DNA databank of sexual assault evidences. The IPDNA performed DNA testing of evidences samples from sexual assaults of caseworks with suspects and with no suspects. All male STRs profiles obtained were inserted in a DNA database. In some cases, there were associations of same males DNA profiles with unsolved cases, showing that there were serial crimes perpetrators. This information was very useful for police investigators to find suspects. The work of forensics scientists with DNA databank and police investigators found out 49 serial rapists that attacked 143 women in the Federal District. 38 out of those 49 serial rapists are already identified and condemned. In 2012 in Brazil, a national law passed created DNA databanks, at state and national levels, of condemned people for violent crimes. The experience of IPDNA will contribute with this new legislation to identify more perpetrators and solve crimes in Brazil.

Theme 3: Mitochondrial DNA analysis

Ancestry evaluation of Sub Saharan male descendant population in Rio de Janeiro inferred by analysis of mitochondrial DNA

R.M.S.M. Hermida [1], P.M. Domingues [1], D.A. Silva [1,2], E.F. Carvalho[1,2].

[1]*Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*

[2]*Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*

The Brazilian population derives from the admixture between Native Amerindians, Europeans, mainly Portuguese, and Africans, mostly Bantu speakers, who were brought to the country as slaves between centuries XVI and XIX. The historical records tell about the port of shipment to America, but lack information about the regions of Africa from where the slaves were taken. In the present study, we aimed to characterize the African maternal genetic-pool of a sample from Rio de Janeiro (Brazil), composed of unrelated men, self-identified as sub-Saharan African descendent. The analysis of mitochondrial DNA control region in 65 self-declared afro descendant revealed a very important African contribution (81%) represented mainly by the L0, L1, L2 and L3 haplogroups, followed by Amerindian (14%) represented by the haplogroups A, C and D, and a lower number of European lineages (5%). Pairwise genetic distance analysis showed significant differences from other Brazilian regions. On the other hand, the sample is genetically close to African populations, especially to the Bantu-speakers. Results suggest that the formation of the population from Rio de Janeiro had great African contribution, followed by Amerindian contribution, both representing the main maternal source in the composition of this population. Moreover, the genetic results obtained are in accordance with the historical facts, which show that the main entrance of the slave trade in Rio de Janeiro occurred from some countries of African continent, as Angola, since they show a great contribution in the formation of the Brazilian population.

Financial support: CNPq

Mitochondrial DNA HV1 and HV2 Variation in Tajikistan, Central Asia

I. Ovchinnikov[1], M. Malek[1], K. Drees[1]

[1]*University of North Dakota, Grand Forks, ND, USA*

Tajikistan is a country in the mountains of southeast Central Asia. Due to its isolation, mtDNA variation in the Tajiks has been fragmentary studied on a limited number of samples. In 1997 saliva samples were collected from unrelated Tajiks across Tajikistan. After long-term preservation DNA was extracted from 2 mm FTA discs. Due to degradation mtDNA was amplified using the primary and secondary PCRs with nested primers. The 91 sequences of the mtDNA hypervariable regions 1 and 2 (HV1 and HV2) belonged to 85 different haplotypes defined by 132 transitions and 9 transversions in 141 of 711 variable sites. The Tajiks demonstrated the nucleotide diversity of 0.0184 ± 0.0092 and the mean number of pairwise nucleotide differences of 13.12 ± 5.96. These data correspond to other populations from Central Asia. The origin of mtDNA lineages traced from western Eurasia (62.6%), eastern Eurasia (25.3%), south Asia (11.0%), and North Africa (1.1%). Significant population structure in the distribution of these mtDNA lineages was revealed within the regional groups in Tajikistan. Pairwise Fst comparisons and the correspondence analysis revealed non-significant differences between the Tajik and Uzbek populations. Although both nations speak languages belonging to different linguistic groups, this result corresponds to their cultural and economic proximity. Surprisingly, after the Uzbeks, the Tajik mtDNA pool most closely resembles to the Ossetians, an Indo-Iranian people from the North Caucasus. The Tajiks also display gene flow and admixture with some other populations of Central Asia and the Iranian Plateau belonging to different linguistic groups.

PCR/ESI-TOF-MS based method for detecting polymorphisms of base compositions of mtDNA in Chinese Han population and testing of maternity duos

L. Li[1], Y. Liu[1], Y. Lin[1], C. Li[1], S. Zhang[1], S. Zhao[1]

[1]*Shanghai Key Laboratory of Forensic Science; IFS, Ministry of Justice, China*

Objective: Polymorphisms of base compositions of hypervariable regions (HV1 and HV2) of mitochondrial DNA were detected in Chinese Han population by PCR/ESI-TOF-MS based method and the assay was applied to maternity testing. METHOD: Base compositions of 12 amplicons in HV1 derived from primers covering coordinates15924-16428 and base compositions of 12 amplicons in HV2 derived from primers covering coordinates 31-576 were determined using mass spectrometry-based PLEX-ID system. The polymorphisms of mtDNA among Han population in eastern China were investigated. The typing method was applied to a special duo case in which a false mother could not be excluded by typing a comprehensive panel of autosomal STRs (46 loci in total). RESULTS: Base compositions of eight segments in HV1and ten segments in HV2 were found to be polymorphic. Highly polymorphic information could be got in 16124-16250 (HV1) and 138-340 (HV2). After the assay was applied to above-mentioned case, the false mother was excluded as the biological mother based on the different mitochondrial profiles of the two subjects despite the fact that they shared an allele at 46 distinct autosomal STR loci.

Conclusions: The mass spectrometry-based method for mitochondrial profiling could have promising prospects. It could importantly supplement the use of autosomal STR loci when evaluating familial associations of closely related individuals.

Analysis of eight mtDNA coding region polymorphisms for characterization of the female lineages ancestry in Alagoas, Brazil

G.C.Cardoso[1], I.P.A. Vasconcelos[1], A.L.T.M. Brandão[1], L.A.F. Silva[1], D.A. Azevedo[1]

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

The analysis of the mitochondrial DNA (mtDNA) polymorphisms has been used in population ancestry studies. A number of multiplexes to minisequencing with SNaPshot kit (Applied Biosystems – USA) have been published in recent years, some of which consists of a large number of markers featuring high resolution with a high cost. In this work we developed a multiplex with eight mtDNA coding region polymorphisms to identify lineages of African, Amerindian and European origin in Alagoas, Northeastern Brazil.

The sample analyzed included 113 individuals of the State of Alagoas, whose haplogroups were previously identified by sequencing of the HVI and HVII regions (Barbosa et al, 2008). The PCR multiplex included the following polymorphisms: 8281-8289d; 1736; 13263; 4883; 3594; 10873; 10400; and 12705. The deletion was detected by polyacrylamide gel electrophoresis (6%) silver stained, and the SNPs were analyzed by multiplex minisequencing with SnaPshot.

The following haplogroups were identified: A (n=13); B (n=4); C (n=13), D (n=3); L3 (n=21); L(xL3) (n=29); M (n=1); and N (n=2). The haplogroups identification by minisequencing was compared to that made by HVI and HVII sequencing. Agreement was observed in 112 of the 113 (99,12%) individuals analyzed. Only an individual identified as M in this work had previously been identified as R by analysis of the HVI and HVII sequences. The results obtained suggest that the markers set used in this work can be used in studies of ancestry in Brazilian populations. Despite not having high resolution this methodology is a low-cost option.

MtDNA investigation of the Cayapa (Chachi) of Ecuador, an indigenous population with high record of the American founder lineage D4h3a

M. Bodner[1], G. Huber[1], B. Zimmermann[1], C. Xavier[1], R. Würzner[2], W. Parson[1,3]

[1]Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria

[2]Division of Hygiene and Medical Microbiology, Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria

[3]Penn State Eberly College of Science, University Park, PA, USA

Native American mtDNA diversity is characterized by four common "pan-American" haplogroups and several minor lineages with limited, sometimes enigmatic dispersal. After the rough picture of American colonization has been clarified in the last decades, the focus of Paleo-Indian mtDNA population genetics has moved towards high-resolution analyses of geographically restricted areas, single tribes, and minor or local lineages that may convey more detailed insights on (additional) migratory waves and routes in the peopling of the Americas.

We here pursue this approach by investigating the mtDNA composition of the Cayapa (Chachi) from Northern Ecuador, for which an Amazonian origin, migration into the Andean highlands and later the Cayapa River basin to evade Incas and Europeans have been suggested. The Cayapa have remained stable in size, and little admixture is reported despite close proximity to African-American population groups since the 19th century. With 120 mtDNA sequences generated according to highest forensic standards, we present the first large complete Cayapa mtDNA control region sample, augmenting the scarce Ecuadorian reference mtDNA data. Haplogroup D4h3a, first reported in 1999 as the "Cayapa lineage", has meanwhile been confirmed as American founder.
Our dataset contributes to the refinement of the phylogeny and phylogeography of this otherwise rare haplogroup that is present at a high proportion in the Cayapa and reveals unobserved variation also within other founder lineages. These findings confirm the necessity of further sequencing efforts at a regional scale to yield the complete picture of American pioneer colonization.

This work received support from the Austrian Science Fund (FWF): L397 and the Theodor Körner Fonds zur Förderung von Wissenschaft und Kunst.

Forensic implications of mitochondrial DNA diversity among Australian Aboriginal people

N. Nagle[1], R.A.H. van Oorschot[2], P. McAllister[3], S. Wilcox[4], R. Turakulov[4], R.J Mitchell[1] & The Genographic Consortium

[1]La Trobe University, Melbourne, Victoria, Australia

[2]Victorian Police Forensic Services Department, Melbourne, Victoria, Australia

[3]Griffith University, Queensland, Australia

[4]Australian Genome Research Facility, Melbourne, Victoria, Australia

Genetic structure and diversity have implications in the creation of DNA databases, especially in cosmopolitan populations such as Australia. The initial colonisation of Australia by the ancestors of contemporary Aboriginal people some 50,000 years ago and their subsequent isolation and hunter-gatherer lifestyle will have had a major impact on their genetic structure. However, our knowledge of genetic variation in Australian Aboriginal peoples is extremely limited compared with other population groups.

Mitochondrial (mt) DNA is maternally inherited, haploid and therefore the lineages retain the chromosome's evolutionary history. Many mitochondrial haplogroups are unique to Australian Aboriginal people (M42a, S, and O), others are unclassified (M and N types) and yet others are shared with a very small number of neighbouring groups (P and Q). Most forensic mtDNA databases, however, comprise hypervariable segment (HVS) sequences only.

We investigated mtDNA variation in ~ 400 samples of self-declared Aboriginal Australians from previously poorly sampled locations. Many of their HVS sequences could not be assigned to a haplogroup with any confidence using a variety of haplogroup predictor software. Many in fact were assigned to a Eurasian haplogroup. Subsequent SNP analysis and Next Generation Sequencing (NGS) of the whole mt genome confirmed these individuals had an indigenous lineage. Our findings suggest caution be applied if using mtDNA HVS sequences only to identify the ethnic affiliation of a sample or human remains. At the very least, SNPs that identify indigenous mt haplogroups should be assayed.

Support for this project was provided by The National Genographic Project and IBM.

Origins and affinities of Australian Aboriginal peoples' – evidence from mitochondrial haplogroup P

N. Nagle[1], R. A.H. van Oorschot[2], P. McAllister[3], S. Wilcox[4], R. Turakulov[4], R.J Mitchell[1] & The Genographic Consortium

[1]La Trobe University, Melbourne, Victoria, Australia

[2]Victorian Police Forensic Services Department, Melbourne, Victoria, Australia

[3]Griffith University, Queensland, Australia

[4] Australian Genome Research Facility, Melbourne, Victoria, Australia

Australian Aboriginal people have one of the oldest continuous cultures outside Africa, with their ancestors arriving in the ancient landmass of Sahul (present day Papua New Guinea (PNG) and Australia) ~50,000 years ago. Limited genetic studies have demonstrated both the uniqueness and antiquity of Australian Aboriginal Y and mtDNA lineages. Mitochondrial (mt) DNA is maternally inherited, haploid and therefore lineages retain the chromosome's evolutionary history.

The most frequent, oldest and most widespread haplogroup in Australia is haplogroup P which is also found in PNG and offshore islands plus Negrito groups in Philippines and is ~60,000 years old. Its origin is controversial – it may have evolved just prior to the first colonisers reaching Sahul. The initial colonisation of Australia by the ancestors of contemporary Aboriginal people some 50,000 years ago and their subsequent isolation and hunter-gatherer lifestyle will have had a major impact on their genetic structure.

Here we report the whole mt genome sequence for 25 new individuals from locations previously poorly or never sampled. These were added to the 35 P sequences in Genbank from all populations, giving a total of 60 sequences. Analysis revealed new P subtypes that are uniquely Australian and these have been dated. Additional examples of P subtypes allowed more accurate dating of these lineages and identification of key SNPs for those subtypes. We also report the first mt sequence from the island of Tasmania, which is dated >30,000 years old.

Support for this project was provided by The National Genographic Project and IBM.

Mitochondrial DNA population data of HV1 and HV2 sequences from Turkish individuals

H. Altuncul[1], O. Bulbul[1],O. Karataș[1], A. Katoglu[1], Y. Demirbas[1], G. Filoglu[1]

[1] Institute of Forensic Sciences, Istanbul University, Turkey

On the forensic cases mitochondrial DNA (mtDNA) analysis can be used with highly degraded samples or low-copy number materials because of its special features. The non-coding region is called the control region which contains hypervariable regions I (HV1) and hypervariable regions II (HV2). Control regions have a lot of variations among different individuals and these are ideal for identification. The aim of this study was to determine the mitochondrial DNA polymorphism in Turkish population. We collected blood samples from unrelated 107 individuals from Turkish populations. The samples were sequenced using the ABI PRISM Bigdye Terminator v3.1 Cycle Sequencing Kit. HV1 region was aligned from 16024 to 16422 and HV2 region was aligned from 73 to 379. Sequences were compared to the revised Cambridge Reference Sequence (rCRS) using SeqScape Version 2.7. Mitochondrial DNA sequences of the HV1 was determined for 102 and HV2 was determined for 107 unrelated Turkish individuals. The most frequent mtDNA haplotype on HV1 region (16183a>C 16189t>C) was shared by 5 individuals. The most frequent haplotype on HV2 region (263a>G 310t>C 310-311insTC) was found in 7 individuals.

The haplotypes diversity and random match probability were calculated to be 0.99 and 0.02 respectively. According to the results in Turkish population on HV2 profile most common polymorphic position were 152t>C, 263a>G, 310t>C,310-311insC, 310-311insTC and 315insC. The HV1 profile most common polymorphic position were 16126t>C, 16183a>C, 16189t>C, 16223c>T and 16294c>T.

Mitochondrial DNA characterization of the human population of East Timor (Timor-Leste)

S.M. Gomes[1], L. Souto[1], B. Zimmermann[2], G. Huber[2], C. Strobl[2], A.W. Röck[2], M. Bodner[2], F. Côrte-Real[3], W. Parson[2,4]

[1]Department of Biology, University of Aveiro, Campus de Santiago, Aveiro, Portugal, Cencifor Centro de Ciências Forenses, Portugal.

[2]Institute of Legal Medicine, Innsbruck Medical University, Müllerstrasse 44, Innsbruck, Austria.

[3]Faculty of Medicine, University of Coimbra, Coimbra, Portugal, Cencifor Centro de Ciências Forenses, Portugal.

[4]Penn State Eberly College of Science, University Park, PA, USA.

Little is known about the human maternal gene pool of East Timor. Here, we investigate the genetic composition of the East Timorese human population using mitochondrial DNA (mtDNA) data for the first time in a big sample.

324 East Timor buccal swab samples were analyzed for the entire mtDNA control region (CR) according to highest forensic quality standards. In addition we performed whole mitochondrial genome (WMG) sequencing of eight samples that could not be assigned to described haplogroups by their CR sequence to contribute to the clarification of the mtDNA phylogeny of Island Southeast Asia (ISEA). Forensic and population statistical parameters from East Timor were compared to populations from Southeast Asia, East Asia, Polynesia, Melanesia and Australia.

In total, 29 different mtDNA haplogroups were distinguished based on complete CR analyses. WMG analyses revealed novel mtDNA lineages within haplogroups Q3, R9c1, M21, P1, M73a, and D6a. Genetic comparisons with the surrounding populations revealed significant genetic differentiation of our East Timor sample. Intra- and inter-population indices indicated that the population of East Timor was most similar to those of Nusa Tenggara and the Moluccas. The large number of hitherto undescribed lineages detected in our sample set indicates that the mtDNA phylogeny of ISEA is not yet fully resolved and pinpoints the need for more (WMG) data from this region.

This work was financially supported by FCT and COMPETE, co-funded by the European Community Fund FEDER: PTDC/CS-ANT/108558/2008, by the FCT fellowship SFRH/BD/63165/2009 and EMPOP FWF TRL397.

Resquencing of Australian Aboriginal mtDNA and Y chromosomes

Y. Xue[1], M. Cerezo Fernandez[1], Y. Chen[1], S. McCarthy[1], M.O. Pollard[1], Q. Ayub[1], N. Nagle[2], P. McAllister[3], R.J. Mitchell[2], C. Tyler-Smith[1]

[1]The Wellcome Trust Sanger Institute, Hinxton, UK

[2]Department of Genetics, La Trobe University, Melbourne, Australia

[3]Griffith University, Gold Coast, Queensland, Australia

Modern humans originated in Africa and spread across the rest of the globe 50-70 thousand years ago. The first identified divergence outside Africa was between the ancestors of the Australian Aborigines and some nearby populations on the one hand, and the ancestors of Asians, Europeans and other non-Africans on the other hand. The genetic characterization of this divergence and subsequent events in Australian Aboriginal history before the colonial era remain poorly described. Seven Australian Aboriginal males requested sequencing of their mitochondrial DNA (mtDNA) and Y chromosomes, and we generated high depth Illumina 100 bp paired-end sequence data. The average sequence coverage of the Y is ~15-20x, and of mtDNA ~300x.

The mtDNAs of the samples belong to new branches of haplogroups P (5 individuals), M (1 individual) and O (1 individual). These haplogroups are known from Australia, but the many new variants illustrate their diversity and distinct origins. The Y chromosomes belong to haplogroups C (3 individuals), K* (3 individuals), both previously known from Australia, and M (1 individual). This last haplogroup has a restricted geographical distribution centred on Papua New Guinea, and illustrates a genetic link between Australia and that region. The ~3,000 new Y-SNPs present in these samples are permitting refined estimates of the ancient coalescence times between Australian lineages and those in the rest of the world, and re-examination of the hypothesis of more resent links between Australia and South Asia, originally based on Y-chromosomal similarities.

Screening of mtDNA SNPs in Chinese Han population using pyrosequencing

Z. Ren[1], H.B. Luo[1], F. Song[1], J. Zhang[1], H. Zhu[1], Y.P. Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

Mitochondrial DNA (mtDNA) single nucleotide polymorphisms (SNPs) have been extensively applied as supplement for HV I and HV II sequencing. They have been proved even more effective for detecting highly degraded samples, hair shaft and bone samples. However, some mtDNA SNPs which had high polymorphisms in database could not be used in Chinese Han population due to ethnic differences. The pyrosequencing (PSQ) technology, including universal primer applications and pooling samples, could reduce assay cost and improved efficiency of screening SNPs. In this study, we aimed to screen a set of mtDNA SNPs which have enough polymorphisms in Chinese Han population. We analyzed 18 candidate SNPs and estimated allelic frequencies using PSQ with pooling samples. Our results revealed that there were nine mtDNA SNPs with enough polymorphisms in Chinese Han population. In conclusion, PSQ was an effective method in screening mtDNA SNPs. A total of nine mtDNA SNPs would be used as candidate markers for forensic purpose. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK16B01) and by the National Natural Science Foundation of China (81273349).

Theme 4: Forensic Y STR typing

Characterisation and haplotype analysis of 11 Y-STR loci in ecuadorian population

A. Gaviria[3], M. Sánchez [1,2], G. Morejon[3], M. Vela[3], V. Aguirre[3], G. Burgos[3], A.K. Zambrano[3],C. Paz –y-Miño[2]

[1]Laboratorio de Genética Molecular. Pontificia Universidad Católica del Ecuador.

[2]Instituto de Investigaciones Biomédicas. Universidad de las Américas.

[3]Laboratorio de Genética Molecular, Cruz Roja Ecuatoriana.

This work pretends to study molecular diversity Y STR to increase Ecuadorian Y-STR database. Data obtained were compared to closed populations in order to evaluate their genetic relationship; moreover it could be used in forensic cases involving different paternal lineages and paternity testing in cases where W is less than 99.99%.

This study included 417 Ecuadorian individuals, Extraction was made according to FTA®, PCR amplification was made using PowerPlex Y System® (PromegaCorp), the Capillar Electrophoresis and detection for amplified Fragments was performed on 3100-Avant Genetic Analyzer, GeneMapper® ID V3.2 was used for allele sizing

It has been reported that the Q1a3a sub-group is closed associated with Native American populations. In this study it was found, *62 unique haplotypes showed 100% of probability with Q haplogroup.*

Additionally, seven mutations that comprise single step (gain or loss) were observed in locus DYS385a/b, DYS390, DYS391, DYS392 and DYS389II. All of these mutations occur singly in different father-son pairs.

DYS392 bin 9: allele or artefact?

B. Kokshoorn[1] ,K. van der Gaag[2], A.J. Kal[1] and P. de Knijff[2]

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

[2] Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

The Yfiler Y chromosomal DNA analysis system (Applied Biosystems) is commonly used in forensic casework. Y chromosomal DNA analysis is often a last resort when analysing mixtures where the male component is a (very) minor contributor to the mixture. Several trace samples were identified, all from sexual assault cases, in which an aberrant peak was observed at locus DYS392. This peak, in bin 9 of the locus, could either be an allele or an artefact. For these and future cases, it is essential to evaluate the nature of these peaks before using the resulting electropherograms (EPG's) for comparison.

The EPG's from the case samples were evaluated. The peak at locus DYS392 was called in all EPG's from the samples, within the standard laboratory guidelines for Yfiler EPG interpretation. The morphology of the peak at locus DYS392 was assessed, as well as its position in the bin relative to the other peaks in the EPG.

The reproducibility of the result was tested using both the Yfiler and Powerplex Y23 (Promega) Y chromosomal DNA analysis systems.

In one case, for two samples, the Yfiler PCR products were sequenced using next generation sequencing methodology.

The occurrence and geographical distribution of allele 9 for locus DYS392 was evaluated using online databases and the scientific record.

Our findings support the presence of an artefact rather than an allele at DYS392 bin 9 in the casework samples. Possible explanations for the phenomenon are discussed.

Modelling Y STR stutter and artefacts

J-A Bright[1,2], J Curran[2], J Buckleton[1], C McGovern[1]

[1]ESR Ltd, Auckland, New Zealand

[2]Department of Statistics, University of Auckland, Auckland, New Zealand

Y STR profile interpretation is complicated by the same phenomena that affect autosomal data. Current research is moving towards continuous interpretation strategies, requiring models that underpin the theory behind these methods. In this study we investigate the use of models developed for autosomal STR data in predicting back stutter, double back stutter, forward stutter and stutter-like artefacts in Y STR loci.

Y STR DNA profiles were generated using a commercially available multiplex, CE data analysis performed using Applied Biosystems' GeneMapper™IDv3.2.1 with a detection limit of 30rfu and models fitted in R and MS EXCEL.

Previous work demonstrated that the longest uninterrupted stretch of repeats (LUS) within an allele is a good predictor of stutter ratio (SR) for autosomal loci. The LUS model was shown to be no better as an explanatory variable for SR than allele designation for Y STR data. This differs from the previous autosomal work most likely due to the simple repeat structure of the majority of the Y STR loci tested. Both mean and variance of the stutter and artefact data was modelled using a lognormal distribution. Whilst the majority of the central data appear normal, there are heavy tails not fully described by the model, consistent with previous autosomal data reports.

This work was supported in part by grant 2011-DN-BX-K541 from the US National Institute of Justice.

Development of a 24-plex Y chromosomal STR loci typing system

C. Liu[1],H. Liu[1], Y. Li[1], H. Sun[2], C. Liu[1]

[1] Guangzhou Forensic Science Institute, Guangdong Province Key Lab of Forensic Genetic, Guangzhou, China

[2]Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

Y chromosomal STR (Y-STR) typing is valuable in patrilineal paternity testing and mixture identification in sex assaulted cases. But the existence of common haplotypes among unrelated males will decrease the power of discrimination of Y-STR and the typing system contained more polymorphic Y-STR markers should be developed.

24 Y-STR markers altogether, which include 3 loci (DYS635 and DYS385a/b) existed in current available commercial kits and 21 new loci (DYS531, DYS630, DYS622, DYS552, DYS510, DYS449, DYS459a/b, DYS446, DYS443, DYS587, DYS527a/b, DYS460, Y-GATA-A10, DYS520, DYS557, DYS522, DYS481, DYS570, DYS444) with high polymorphism (GD>0.6) were co-amplified in a system, which is called 24-plex here. The loci were divided into 4 groups with the primers labeled with fluorescence 6-FAM, HEX, TAMRA, and ROX, respectively and the internal size standard labeled with SIZ.

Satisfactory typing results of the 24 Y-STR loci were obtained with the self-developed multiplex system for the 200 unrelated Chinese Han individuals. Repeated analysis of random DNA samples yielded consistent results. 0.313ng input DNA was successfully analyzed. 198 haplotypes were observed in 200 Chinese unrelated individuals and the haplotype diversity was 0.999899, While there were 196 haplotypes observed in the same population with AmpFlSTR Yfiler™ kit and the haplotype diversity was 0.999749. If these two systems were collaborated, 199 haplotypes were obtained and the haplotype diversity reached 0.999950. The number of different haplotypes and haplotype diversity values are indicated in Table 1.The 24-plex Y-STR typing system established in the study is stable and efficient. It can be used in forensic casework and paternity testing.

This study was supported by the National Natural Science Foundation of China (81273347) and Guangdong Key Laboratory of Forensic Genetics (Project No.2010A060801001).

Internal validation study of the PowerPlex® y23 system

C. Gehrig[1], V. Castella[1]

[1]University Center of Legal Medicine, Lausanne – Geneva, Switzerland

Introduction The PowerPlex Y23 System is a 23-loci, 5-color Y-STR multiplex designed for genotyping forensic casework samples, database samples and paternity samples. The kit contains: all 12 loci in the current PowerPlex Y System, the additional 5 loci found in AmpFlSTR Y-filer, plus 6 new loci.

Aims With the aim of replacing the PowerPlex Y system (12 loci), which is in use in our laboratory, with a more discriminating and robust multiplex, we tested the PowerPlex Y23 System. An internal validation study of this latter kit was therefore conducted including the following aspects: sensitivity, mixture studies of male-male and female-male DNA, performance with simulated inhibition, external quality controls and stutter calculations. For the amplification sensitivity study, the performance was compared with the AmpFlSTR Y-filer and PowerPlex Y kits.

Materials and methods The amplifications were performed in a PCR volume reaction of 25ml at 30 PCR cycles according to the protocol of the manufacturer.

Results and discussion A forensic casework will be discussed.

When compared with the PowerPlex Y, the PowerPlex Y23, with its additional loci and increased haplotype resolution, showed improved performances, especially in regards to its sensitivity. Making this kit well suited for a wide range of forensic samples.

Could the PowerPlex® Y23 System resolve the story of the ancient Yakuts?

C. Keyser[1,2], C. Hollard[1], A. Gonzalez[1,2], E. Crubézy[2], B. Ludes[1,2]

[1]Institut de Médecine Légale, Laboratoire d'Anthropologie Moléculaire, CNRS UMR5288, Université de Strasbourg, France

[2]Laboratoire AMIS, CNRS UMR 5288, Université de Toulouse, France

In the framework of a study aiming at determining the ethnogenesis of an Eastern Siberian ethnic group, the Yakuts, we studied skeletal remains from nearly 140 individuals dated from the 15th to the 19th century. Archaeological excavations were performed by the French Archaeological Mission in Eastern Siberia over a period of 10 years (2002-2012) in three regions of Yakutia: Central Yakutia, Vilyuy river basin and Verkhoyansk area. The exceptional preservation state of most of the excavated bodies enabled us to successfully analyze Y-chromosomal, autosomal and mitochondrial DNA. The Y-chromosomal STRs analysis, performed on the male specimens (n = 59) by means of the AmpFlSTR® Y-Filer™ kit (Applied Biosystems) revealed a low genetic diversity (0.75). Indeed, one haplotype was widely shared among our sample set (28/59). The presence of this dominant 17-STR haplotype strongly suggested a strong reduction in male population of Yakuts. In an attempt to increase the Y-haplotype resolution, we decided to test the newly commercialized PowerPlex® Y23 System from Promega, which includes 6 new loci with high gene diversity. DNAs from 26 ancient unrelated Yakut specimens sharing the

dominant Y-haplotype were amplified according to the manufacturer's recommendations and run on a 3500 Genetic Analyser. With the addition of these new loci, only 4 of the 26 samples were distinguished from each other. A detailed analysis of the results is presented as well as some hypothesis explaining why the increased number of Y-STR loci was not enough to completely resolve the story of the ancient Yakuts.

Native American Y- STR haplotyping: its forensic relevance in Argentina

A. Sala[1], C. Bobillo [1], M. Caputo [1], M.C. Martí [2], E. Alechine [1], D. Corach [1]

[1]Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires and CONICET, Buenos Aires, Argentina

[2]Centro Científico Tecnológico, CONICET, Santa Fe, Argentina

The Argentinean population is the result of complex admixture events. Major interactions took place between Native Americans people and European conquerors and immigrants. At present, autochthonous people represent only 2.3% of the extant population. Due to discrimination and poverty these groups tend to live in social isolation and prone to perpetrate criminal acts, as in any other social group. Taking into account that those populations show differential allele or haplotype frequencies, its research is relevant for the forensic interpretation. Aiming to investigate the informativeness of the Y-STR markers, a set of 236 samples of south Amerindian were analyzed, including the ethnic groups: Toba, Pilaga, Wichi and Mocovi, inhabiting Northern and central Argentina. Haplotype analysis comprised YHRD minimal haplotype plus DYS437, DYS438 and DYS439 Y-STRs. The SNP M3-Q3 was typed in order to discriminate the most frequent Amerindian haplogroup. Out of 202 Q1a3a haplotypes, 135 were unique (66.83%), 17 haplotypes were share between different groups with frequencies ranging 0.99% to 10.9%. From these, only three were found out of Argentina, in isolated cases from Native American people or admixed populations in America. None of these haplotypes were found in Argentinean males of European ancestry.

Previous investigation allowed us to find some particular mitochondrial haplotypes that together with Y-STRs information might provide a clue indicative of ethnicity of a sample. Such information might provide the forensic lab about the references databases to be employed for statistical analysis.

A modified multiplex PCR system for 13 RM Y-STRs with separate amplification of two different repeat motif structures in DYF403S1a

E.Y. Lee[1], H.Y. Lee[1,2], W.I. Yang[1], K.J. Shin[1,2]

[1]Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

[2]Human Identification Research Center, Yonsei University, Seoul, Korea

The Y chromosome STRs are very useful markers in forensic science and human genetics. With the 17 Y-STRs currently used in forensic genetics, however, there are some limitations which fail to discriminate between relative males and have low haplotype diversity in some populations. To overcome these limitations, 13 rapidly mutating (RM) Y-STRs, because of their high mutation rate, are suggested as revolutionizing new tool that can wide Y-chromosomal application from paternal lineage differentiation to male individualization. Therefore, we have constructed two multiplex PCR sets for the amplification of 13 RM Y-STRs with amplicon size ranging from 90 bp to 400 bp. In particular, with the developed multiplex PCR system, it is possible to differentiate DYF403S1a (three peaks) into DYF403S1a (two peaks) and DYF403S1b1 (one peak) by difference in their repeat motif structures. Since DYF403S1b1 possesses single nucleotide polymorphisms which are different from DYF403S1a at both the front and rear flanking region of the repeat motif, the two loci could be separately amplified using allele-specific primers. In addition, DYF403S1b of the previous report by Ballantyne et al. (Forensic Sci Int Genet. 2012), which has similar flanking region sequence and repeat motif structure to DYF403S1b1, was renamed DYF403S1b2. The multiplex PCR system of the present study will not only facilitate the analysis of 13 RM Y-STRs using two multiplexes, but also increase the discrimination capacity even more by separate amplification of DYF403S1a and DYF403S1b1.

** We referenced allelic ladder and nomenclature information of RM Y-STRs which were provided by Prof. Kayser and the International RM Y-STR Study Group as part of a multicenter study.*

*** This work was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2011-0027729).*

Study on the genetic polymorphisms of Y chromosomal DNA short tandem repeat loci applied to analyzing the relative affinities among ethnic groups in Taiwan

F.C. Wu[1], M.Y Chen[1], C.H Chao[1], C.E Pu[1]

[1]Department of Forensic Science, Investigation Bureau, Ministry of Justice, New Taipei City, Taiwan, Republic of China.

Taiwan's population of about 23 million is heterogeneous and is made up of 97.9 % Han Chinese and 2.1 % indigenous people. Han Chinese are composed of Minnan (68.9 %), Hakka (15 %) and mainland Chinese (14 %) who arrived from China after World War II.

Use Y-STRs to analyzing the relative affinities among ethnic groups in Taiwan. After obtaining consent, buccal swab samples were collected from 1,281 unrelated male volunteers of 14 ethnic groups residing in Taiwan.

Target DNA (0.1 ng/μL) was amplified by PCR, using the AmpFlSTR® YFiler™ PCR Amplification Kit (Applied Biosystems). Amplification was performed under the following conditions: 95°C for 11 min, followed by 30 cycles at 94°C for 1 min, 61°C for 1 min and 72°C for 1 min, with a final extension step at 60°C for 80 min.

The 1,281 donators included Holo, Hakka, Mainlander, Amis, Paiwan, Atayal, Bunun, Truku, Rukai, Puyuma, Tsou, Saisiyat, Yami and Thao. We got 923 DNA Y-STR haplotypes for identifying paternal affinity.

Allele frequencies for the 17 Y-STRs loci were determined from 1,281 samples. The genetic distance values below 0.2, which show high genetic affinity between compared groups, have four groups: (1) Holo, Hakka and Mainlander; (2) Paiwan and Rukai; (3) Paiwan and Puyuma; (4) Atayal and Truku.

From the relation of genetic blood and geographic environment, we found the Atayal, Bunun, Truku and Tsou tribes had the high frequencies over 78.6 % of allele 13 and the Amis, Paiwan, Rukai, Puyuma, Saisiyat, Yami and Thao tribes had the high frequencies over 76.1 % of allele 12.

Classification of the Y-SNP haplogroup distributions of 90 Western Eurasian populations using a self-learning algorithm

H.Pamjav[1], Z.Juhász[2], F. Tibor[1]and E. Németh[1]

[1]Network of Forensic Science Institutes, Institute of Forensic Medicine, DNA Laboratory, Budapest, Hungary

[2]Research Centre for Natural Sciences of the HAS, Department of Complex Systems, Budapest, Hungary

The understanding historical relationship between populations is a core aspect of human population history studies. We have analyzed the frequency of 18 different Y-SNP haplogroups in 90 Western Eurasian populations. Classification of haplogroup distribution vectors using a new self-learning classification algorithm so called "self organizing cloud (SOC)" proved to be an effective tool to identify population groups (classes) which share common paternal genetic features.

By means of the algorithm, we have determined 10 different classes of populations based on the similarity of haplogroup composition. The analysis showed that paternal genetic markers tend to reflect geographical proximity of populations better than linguistic relationship, although certain Y-SNP haplogroups have relatively good correlation with specific language families. In addition, the separate genetic maps of the language families showed a good correlation between linguistic subfamilies and genetic classifications.

More than half of the studied populations belonged to three geographically neighboring classes which also had the most diverse haplogroup composition containing all the typical Western Eurasian paternal haplogroups. These three main classes may reflect the spread of agriculture and farmers from the Fertile Crescent to the Western (Eastern) Europe and East (Central) Asia.

Further ancient DNA research and more detailed haplogroup data is needed to elaborate the relationship between the genetic results, spread of languages and archaeological cultures more accurately. The correlation of MtDNA haplogroups with geography and linguistics, as well as Y-SNP relationships in East Asia and Africa can be effectively studied in the future with similar methods.

Distribution of Y chromosome haplogroup Q in Greenlanders

J.K. Olofsson[1], C. Børsting[1], N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Frequencies of Y chromosomal haplogroups (Y-HGs) can be used to infer current and historic migrations of humans. The Greenlandic population is characterized by migrations from two major areas: from Siberia through the northern parts of North America and from Scandinavia. HG-P*(xR1-M173)-M45 was previously found in up to 50% of Greenlanders. HG-P*(xR1-M173)-M45 includes HG-Q-M242 that is characteristic of aboriginal individuals of North and South America. The aim of this study was to investigate the distribution of sub-HGs within HG-Q-M242 in the Greenlandic population.

A multiplex of 15 Y-SNPs covering major sub-HGs within HG-Q-M242 were constructed using the AssayDesign software (Sequenom). The 15 Y-SNPs were amplified and analysed on the Sequenom platform according to the manufacturer's recommendations. Two sub-HGs within HG-Q-M242 were found to be prominent in the Greenlandic population, Q- L56/L57(xM19) and Q-NWT01. Q-L56/L57(xM19) is a haplogroup known to be present in Native North Americans. Q-NWT01 is located downstream of Q-MEH2 in the Y-chromosomal tree and has previously been found in high frequencies in Eskimoan-speaking populations of northern Canada. Distribution of sub-haplogroups of HG-Q in Greenlanders will be presented.

Using Y-STRs for exclusion in a case of complex biological relationships

M.L.J Bravo[1], A Manrique[1,2], D.P. Aguirre[1], L Mendoza[1,3], J.J. Builes[1,2]

[1] Laboratorio GENES Ltda, Medellín, Colombia.

[2] Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

[3] Universidad Pontificia Bolivariana, Medellín, Colombia

Currently, laboratories have available a wide range of genetic markers associated with different modes of inheritance and therefore linked to different types of chromosomes and genomes present in humans. When we are challenged with complex cases we have to recourse to a combination of more than one type of markers to achieve the levels required for inclusiveness or the number of incompatible systems for the exclusion of the case investigated. The objective of this research was to provide a scientific solution to a legal dispute over a complex biological parent-child relationship to claim an inheritance of property.

For this study we counted only with the mother, two sisters and a paternal uncle (PUAF) of the deceased alleged father, the alleged son and his biological mother. The research was based on the reconstruction of the alleged paternal grandfather genotype with 15 A-STR from haploid parental genotypes of the alleged father's sisters. Additionally, the PUAF and alleged son were typed with Y-chromosome Minimal Haplotype.

With the genotypes of the two alleged grandparents we only detected a single inconsistency with the child's paternal haploid genotype, which could be interpreted as a new mutation in the germ cells of the alleged father. Only it was a conclusive diagnosis of exclusion of the biological relationship with the study of the Y chromosome Minimal Haplotype, conducted between PUAF and alleged son. These results show once again the efficiency of the sex chromosomes STR loci on complex biological relationship investigations.

Enhancing male identification capabilities with a new commercial 27 loci Y-STR multiplex

K.N. Ballantyne[1,2], M. Kayser[2],C. Bormann Chung[3], J. Mulero[3]

[1] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Victoria, Australia

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

[3] Life Technologies, Foster City, United States of America

Whilst the current set of 17 highly diverse Y-STRs in the AmpF*l*STR Yfiler panel performs well for many forensic applications, it can fail to resolve male lineages, and is unlikely to differentiate between both close and distantly related males. To rectify these issues, a new commercial 27 Y-STR multiplex has been developed by Life Technologies. Full concordance with existing Y-STR haplotype databases is maintained through the inclusion of all 17 Yfiler Y-STRs, while the 10 new Y-STRs, including 7 rapidly mutating Y-STRs, provide enhanced power for male lineage and relative differentiation. With this new multiplex the probability of father-son differentiation is increased by about 3.5 times relative to Yfiler (from 0.041 to 0.142) providing a theoretical limit of 7 generations of males sharing identical 27 Y-STR haplotypes. Furthermore, the increased haplotype diversity of the panel results in a significant decrease in haplotype sharing between unrelated males; the number of singletons in a global database is increased by 1.16-fold and the total number of haplotypes by 8%. The inclusion of 18 Y-STRs with medium mutation rates allows the detection of related individuals for familial searching and paternity testing, whilst the inclusion of 7 rapidly mutating Y-STRs allows adventitious matches to be reduced, and at least distant family members to be distinguished. The unique combination of Y-STRs in this new commercial multiplex will enhance the practical

relevance of forensic Y-chromosome analysis by substantially reducing the caveats placed on patrilineal genetic evidence.

Comparison of DNA typing using AmpFlSTR Yfiler and PowerPlex Y System for specimens subject to very long storage

K. Tsukada, Y. Harayama, Y. Itoga, M. Shimizu, Y. Kurasawa, K. Kasahara

Department of Forensic medicine, Criminal investigation lab., Nagano. Pref. Police H.Q., Nagano, Japan.

Autosomal DNA typing is a powerful and highly discriminating tool used in criminal investigations and forensic medicine and for identification following earthquakes, tsunamis, and other natural disasters. Y-chromosome DNA typing, in contrast, is often applied to obtain supplemental information, due to the method's lower power of discrimination. Nevertheless, since the Y-chromosome is male-specific, Y-STR DNA typing is useful in paternity testing and sexual crime investigations. With the lifting of various statutes of limitations, the need for DNA typing of degraded specimens has grown in recent years. This underscores the importance of understanding the capabilities and characteristics of specific Y-STR typing kits.

Our study compared the amplification efficiency of AmpFlSTR Yfiler and PowerPlex Y System using extracted DNA from blood stains kept in very long-term storage.

The DNA was extracted from bloodstains stored for 22 to 30 years at room temperature. DNA typing was performed using AmpFlSTR Yfiler and PowerPlex Y System.

The AmpFlSTR Yfiler tended to provide better results than PowerPlex Y System for DNA typing of degraded specimens.

Sequencing-based Characterization of Y-STR Variants in Korean Population

K.C. Park[1], S-Y. Kim[2], S.J. Oh[1], Y. Jung[1], K.M. Woo[2], M.H. Kang[1], H. Koo[1], S.H. Lee[2]

[1] Medical Genomics Research Center, KRIBB, Daejeon, Republic of Korea

[2] DNA Forensic Division, Supreme Prosecutor's Office, Seoul, Republic of Korea

We performed Y-STR haplotype analysis of 6300 Korean males by using an AmpF/STR® Yfiler™ kit and found that there were 348 total allelic variants of 45 types in 15 Y-STRs which lead to inaccurate personal identification by the commercial kit. To characterize the variations, we selected 41 variants that were either not previously identified (data from www.cstl.nist.gov/strbase/) or occurred at high frequency, and performed PCR assays for the selected variants using in-house primers. PCR amplicons were directly sequenced for single allelic STRs or after cloning into T-vector for bi-allelic STRs. The microvariations included a nucleotide insertion or deletion in the middle of or at the end of core structures. Interestingly, 19.1 allele of DYS385 has a 22-nucleotide-long insertion, whose sequence was identical to that of the upstream flanking region, suggesting that slipped-strand mispairing may have occurred during DNA replication. We also confirmed other microvariants whose repeat numbers were not allocated yet in DYS438, DYS456, DYS458, and DYS635. Moreover, we found that there is a dinucleotide deletion outside the core STR region of the sample that has been reported as a null allele in DYS390; this may be responsible for the failure of primer binding. Furthermore, using PCR analysis of sequence-tagged sites (STS) markers, we specified regional deletions in the AZFb region of DYS385 and DYS392 null alleles and AZFc region of DYS448 null alleles. Taken together, our results suggest that analysis of sequence features of STR variants may essential for increasing the discriminatory power of STR typing kits.

Study of rapidly mutating Y-STRs in a Portuguese population

P. Brito[1,2], M. Carvalho[1,2], V. Bogas[1,2], A.M. Bento[1,2], A. Serra[1], L. Andrade[1,2] , M. São-Bento[1,2], L. Souto[2,4], F. Corte-Real, F.[2,3], M.J. Anjos[1,2]

[1] Forensics Genetic Service, Central Delegation, National Institute of Legal Medicine and Forensic Sciences, I.P., Coimbra, Portugal

[2] Cencifor, Forensic Science Centre, Portugal

[3] National Institute of Legal Medicine and Forensic Sciences, Portugal

[4] Department of Biology, University of Aveiro, Portugal

In forensic genetics the study of Y-STRS is normally used as a lineage marker due to his block transfer property through generations. However, in certain forensic caseworks, it could be an advantage to be able to discriminate two samples belonging to different individuals from the same parental lineage, based on analysis of Y chromosome. New Y-STRs have recently been discovered, with surprisingly high mutation rates when compared with the Y-STRs commonly used in genetic forensics. These markers (DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a + b, DYS626,DYS627, DYF403S1a + b, DYF404S1, DYS449, DYS547 and DYS612), denominated rapidly mutating Y-STRs (RM Y-STRs), promise to be an important tool in the differentiation of two male individuals closely related. After a collaboration project with the RM Y-STRs Study Group, to which we contributed with the study of a population from Angola, we now present the results of another population study from Portugal (N=100), contributing to the increase of the worldwide database, relatively to these markers.

Forensic significance and population structure of some Nigerian populations Y-STR haplotypes.

C. L. Cole-Showers[1, 2], S. Davison[1] and M. E. D'Amato[1]

[1] Forensic DNA Laboratory, Department of Biotechnology, Faculty of Natural Science, University of the Western Cape, Modderdam Road, Bellville 7535, Republic of South Africa.

[2.] Department of Biochemistry, Faculty of Science, Lagos State University, Badagry Express Road, PMB 1087, Apapa, Lagos, Nigeria.

Forensic DNA analyses offer fast and precise methodologies that could be easily deployed for the resolution of sexual assault cases and others with limiting DNA concentration. This however requires a good database. The 11-loci SWGDAM recommended Y-STRs were evaluated in Nigerian populations. Forensically significant parameters and population differentiations were also determined and discussed in the broader context of Africa.

Genomic DNA was extracted from 465 unrelated men representing Afro-Asiatic (Hausa) and Niger-Congo (Igbo, Yoruba, Edo and Ijaw) linguistic groups. The STRs were amplified in a single multiplex PCR. The haplotypes were determined with an ABI 377 sequencer and associated software. Allelic frequencies (AF), Genetic Diversity (GD), Discrimination Capacity (DC), Haplotype Diversity (HD) and Random Match Probability (RMP) were calculated. Population structure was analyzed using Principal Component Analysis (PCA) and hierarchical Analysis of Molecular Variation (AMOVA). Nigeria dataset was compared with published data for Tunisia, Angola, Uganda, Burkina Faso and Cameroon.

The studied Nigerian populations showed 49 and 344 shared and unique haplotypes respectively with the DC =0.84516, HD =0.9989891 and RMP =0.0010109. The markers showed DC < 0.85 in the African countries evaluated.Loci DYS385ab and DYS392 showed the highest

(0.91544) and lowest (0.29359) GD respectively. Edo population (HD=0.9998671) was the most diverse while Ijaw (HD= 0.9904708) was the least. Fine scale sampling over a broad geographical area revealed Hausa population is not homogeneous. Linguistic barrier (Fst = 0.07451, P< 0.00001) is a stronger driving force for Nigerian population structure.

This work was funded by the National Research Foundation of South Africa and the UWC.

Evaluation of the forensic relevance of two Y-STR genotyping systems in the Botswana population

T. Tau[1], S. Davison[1], M.E. D'Amato[1]

[1]Forensic DNA Laboratory, University of the Western Cape, Cape Town, South Africa.

There is a scarcity of Y-STR data available on African populations. The aim of this study was to study two forensically relevant Y-STR systems in population samples from Botswana: (1) 17 markers of AmpFlSTR® Yfiler® (Applied BioSystems) and (2) an in-house multiplex designed based on its forensic benefit to South African populations, the UWC 10-PLEX [1]. Buccal swabs were collected from 176 Botswana male students at universities in Cape Town (South Africa) and Gaborone (Botswana). DNA was extracted from the buccal swabs and the 27 loci were amplified in three multiplex reactions. Analysis was done using the ABI 3500 Genetic Analyzer. Statistical analyses were performed to evaluate gene and haplotype diversity using Arlequin 3.1, and lineage composition was analysed using STRUCTURE 2.3.4. For Yfiler, the discrimination capacity (DC) was 0.9602 and haplotype diversity (HD), 0.99881. For UWC 10-PLEX, the DC was 0.9545 and the HD 0.99886. Paired t-test to compare the discrimination capacity of the two systems was not significant (t = 0.5762, p = 0.5653). STRUCTURE analysis identified two genetic clusters using Y-filer, and 4 main clusters using the UWC 10-PLEX. Both systems show similar efficacy for forensic application. However, the loci found in the UWC 10-PLEX are more informative for analysis of the population structure in the Botswana population.

This work was funded by the National Research Foundation of South Africa and the UWC.

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Rapidly Mutating Y-STRs Multiplex Genotyping Panel to Investigate UAE Population*

R. Alghafri[1,2], W. Goodwin[1], S. Hadi[1]

[1]University of Central Lancashire, Preston, Lancashire, United Kingdom

[2]Dubai Police General Head Quarters, Dubai, United Arab Emirates

Y chromosome short tandem repeat (Y-STR) profiling has been broadly applied in forensic casework in sexual assault cases where male/female or male/male mixtures are expected and also for population studies, genealogical research and kinship analysis (Roewer 2009). Recently rapidly mutating Y STRs were described (Ballantyne et al. 2012). These loci are expected to help investigating inbred populations and also differentiating closely related males. We have developed a multiplex comprising of 13 rapidly mutating Y STRs (RM Y-STR) that can be amplified simultaneously. The multiplex will aid investigating the human genetic structure of United Arab Emirates (UAE) populations and would also be used to investigate unresolved forensic cases in Dubai Police Forensic Department. Thirteen, simultaneously amplified, markers included in multiplex are: DYF387S1, DYF339S1, DYF403S1ab, DYF404S1, DYS449, DYS528, DYS526ab, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627. Two primer sets for DYF387S1 and DYS570 loci have been redesigned to accommodate the loci within the multiplex using 5-dye chemistry. The alleles amplified using newly designed primers were sequenced to confirm the amplification of desired products. A mini validation of the new multiplex has been carried out including specificity, sensitivity and mixture studies. 13 RM Y-STR markers have been analysed in 600 male samples from UAE population. Allelic frequencies, haplotype diversity and discrimination capacity were determined for the 13 RM Y-STRs. Mutations pattern analysis of the RM Y-STR loci in a typical UAE family has been carried out and will be presented.

**This research project funded by Dubai Police General Head Quarters*

Development and Validation of a Next Generation Y-STR Multiplex for Forensic Applications

C. Bormann Chung[1], S. Gopinath[1], J. Muehling, A. Carbonaro and J. Mulero

[1]Life Technologies Corporation, USA

Y-chromosomal markers have proven useful in solving investigations where low levels of male DNA are present in a high female DNA background. An intrinsic limitation of Y-STRs compared with autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome. Thus, in an effort to increase the power of discrimination we have developed a new 27-plex Y-STR system that includes the 17 markers from the AmpFlSTR Yfiler kit plus 10 additional highly polymorphic Y-STR markers (DYS576, DYS627, DYS460, DYS518, DYS570, DYS449, DYS481, DYF387S1a/b and DYS533). These ten new loci include 7 rapidly mutating Y-STR loci which allow for improved discrimination of related individuals.

The new multiplex is a dual application assay designed to amplify DNA from extracted casework samples and database samples from storage cards and swab lysates via direct amplification. Compared to the previous Yfiler® Kit, the new multiplex shows improved performance in inhibited samples, admixed male and female samples at ratios >1:1000 and better differentiation in male:male mixture samples in high female DNA background. Additionally under optimized conditions, no reproducible cross-reactive products were obtained on bacteria and commonly encountered animal species. The haplotype diversity and discriminatory capacity calculations for several population groups will be presented, as well as father-son studies and validation studies demonstrating improved performance with challenging samples.

Powerplex® Y 23 System: molecular characterization of a null allele at locus DYS549

S. Turrina[1], S. Caratti[1], D. De Leo[1]

[1]Department of Public Health and Community Medicine, Institute of Legal Medicine, Forensic Genetic Laboratory, University of Verona, Verona, Italy

We observed a null allele pattern at locus DYS549 in a male individual from North-East Italy typed with the PowerPlex® Y 23 System (Promega).

To investigate whether this pattern was due to the presence of a primer binding site mutation or a microdeletion within the primer binding sites or on the locus target region, the sample was amplified with newly designed external DYS549 primers obtained from GenBank sequence (GDB: 11503916). Sequencing of the man's allele was carried out with the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems), according to the manufacturer's instructions. Sequences were run on the ABI PRISM 3130 Genetic Analyzer (Applied

Biosystems) and analyzed using the Sequencing Analysis v.5.3.1 and the Seqscape v2.6 softwares (Applied Biosystems). An additional amplification with fluorescently-labelled forward DYS549 primer was also performed.

A normal hemizygous genotype at this locus was generated by the PCR amplification with the new external primers thus indicating the presence of a point mutation in the binding site of the original primer set of Powerplex® Y 23 System (Promega), further confirmed by sequence analysis. Re-extracting and re-amplifying the sample twice corroborate these results. Considering that DYS549 marker has been included for the first time in a Y-STR multiplex kit, it may be recommendable ascertainment of dropout observation in order to perform a statistical evaluation of the occurrence.

Y-Chromosome Lineage in Five Regional Mongolian Populations

T. Yamamoto[1], T. Senda[1], D. Horiba[1], M. Sakuma[1], Y. Kawaguchi[1], Y. Kano[1]

[1]Nagoya University, Nagoya, Japan

Introduction It is said the Y-chromosomal diversity is lower around Mongolia, from northern east to central Asia than the other areas of the world due to the observation of "star" cluster chromosome, likely to be originated from the male-line descendants of Genghis Khan.

Aims Y-STRs and Y-haplogroups (Y-HGs) were analyzed to investigate the genetic diversity in Mongolian male lineage and the paternal genetic relationship between Mongolian and Japanese.

Materials and methods DNA samples collected from 5 regional Mongolian populations (Ulaangom, Ulaanbaatar, Dalandzadgad, Undurkhaan, and Choibalsan; N=493) were analyzed for 17 Y-STRs using a Yfiler kit, and for Y-HGs mainly by a method using a SNaPshot multiplex kit (Geppert, FSI genet.5:100, 2011). We constructed a phylogenetic network based on the Y-STRs haplotypes with the Y-HGs and region information using the program Network 4.6.1.1 mixed with 138 Japanese male data.

Results and discussion We observed 350 and 132 haplotypes in Mongolian and Japanese males, respectively. The haplotype diversity and discrimination capacity were 0.9934 and 0.7099 in Mongolian, and 0.9989 and 0.9565 in Japanese, respectively. These values shows low diversity in Mongolian males. About 63% of Mongolian males had C3 groups from 50% in Ulaanbaatar to 69% in Ulaangom. The 96% of C3 was C3*, which is C3 except C3a to C3f, in Dalandzadgad, meanwhile the 78% of C3 was C3c in Ulaangom. The network analysis revealed that at least 4 major "star" or "star"-like cluster exist although the degrees of influence were variable. These clusters suggested that at least 4 major male ancestors with Y-HG-C3 have affected the gene pool of Mongolian males at the different periods.

Validation of a novel Y-SNPs multiplex system for forensic application

D. Zhou[1], J. Yan[1], D.Z.Tang[2], L.C.Liao[1], Y.B.Wang[2], Z.Wang[1], Y.P. Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

[2]China National Accreditation Service for Conformity Assessment, Beijing100062, Beijing, China

The single nucleotide polymorphisms on the Y chromosome (Y-SNPs) have been considered to be particularly valuable in forensic casework. However, Y-SNPs were mostly population specific and lacked biallelic polymorphisms in the Asian population. We previously established a novel Y-SNPs multiplex system for the Chinese Han population using Pyrosequencing technology and SNaPshot method. The system consisted of 20 Y-SNP markers that were highly polymorphic and of value to establish haplotypes in the Chinese Han population. In the present study, validation of the multiplex system, including sensitivity, degraded DNA, species specificity and mixture study, were performed according to the FBI/SWGDAM guideline. Our results demonstrated the minimal amount of DNA input with which a complete SNP profile could be detected was 300pg. Using the multiplex system, the reliable result of Y-SNP typing could be obtained even from degraded DNA. No corresponding SNP profiles were observed in the forensically relevant animal species. Additionally, the male-female mixture study showed that female DNA did not influence the results of Y-SNP typing even in the background of more than 500-fold excess of female DNA. In conclusion, the novel Y-SNPs multiplex system could be used as a powerful tool for forensic applications in the Chinese Han population. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK26B06) and by the National Natural Science Foundation of China (81273349).

A Prototype Y-STR Multiplex PCR System to Improve DNA Typing Performance with Forensically Relevant Samples

Y.N. Oh[1], E.Y. Lee[1], H.Y. Kim[2], S.J. Park[2], Y.G. Yang[3], K.J. Shin[1]

[1]Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

[2]DNA Analysis Laboratory, DNA Forensic Division, Supreme Prosecutors' Office, Seoul, Korea

[3]DNA BioQuest, Inc., Seoul, Korea

Y chromosomal short tandem repeats (Y-STRs) are often used to identify male DNA profile in forensic casework, such as sexual assaults, paternity test and male lineage investigation. Here, we present a prototype Y-STR multiplex PCR system, Kplex Y17 system, which was designed to improve DNA typing performance on various forensically relevant samples. The Kplex Y17 system amplifies DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385ab, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, and Y-GATA-H4 loci in the AmpFlSTR® Yfiler™ PCR amplification kit (Yfiler kit, Applied Biosystems). The amplicon sizes of large-sized (> 200 bp) Yfiler loci including DYS389I/II and DYS385ab were reduced to mini- or midi-sizes. The Kplex Y17 system has sensitivity to get full profile from 100 pg of male DNA with 30 thermal cycles and shows specificity to analyze male DNA profile from mixed male/female DNA in a ratio of 1:1000 without any interference or reduction of signal. In addition, allelic range was expanded to cover most of the off ladder alleles reported in Koreans and the PowerPlex® Y23 system (Promega), and the interval between loci was adjusted to be at least 10 bp even in case rare allele exists. Therefore, the developed Y-STR multiplex system will be a real complement to the Yfiler kit by enabling researchers to obtain DNA typing result at large-sized Yfiler loci on challenged samples.

** This work was supported by the research project for practical use and advancement of forensic DNA analysis of Supreme Prosecutors' Office, Republic of Korea (1333-304, 2012).*

Y chromosome interstitial deletions of a father and his son: a systematic case analysis

Y. Gao[1], Y. He[1], X. Gao[1], J. Wan[1], C. Wang[1], X. Zhao[1]

[1]Department of Forensic Medicine, Medical College of Soochow University, Suzhou, Jiangsu, P.R. China

Introduction: Amelogenin (AMEL) and Y-chromosomal short tandem repeats (Y-STRs) have been used as routine tools for gender detection in forensic DNA analysis. These markers may cause misinterpretations in forensic casework due to point mutations or microdeletions. In an

accidental traffic case, a standard paternity test including mother and son was performed to identify the victim (suspect father). The results support the biological relationship between the deceased father and the son. However, AMELY dropout was noticed for the son while the deceased father seemed normal on AMEL locus.

Aims: To investigate potential causes of AMELY dropout for the son.

Materials and methods: DNA samples of the deceased father and his son were analysed using AmpFLSTR® Y-filer™ PCR Amplification kit. Additional 30 sequence tagged sites (STSs) were used to assess and estimate the size of the breakpoint regions on the Y chromosome.

Results and discussion: The alleles of the 16 Y-STRs were thoroughly detected for the father. As for the son, however, expression of DYS456 and DYS393 were detected only. Additional Y-STSs analysis revealed interstitial deletions for both the father and the son. The deletion's length was estimated to be in the range of 4.14-6.10 Mb for the father and 22.7-53.4 Mb for the son, respectively. As far as we know, this is the first report which has systematically analysed a father-son pair regarding Y-chromosome deletions in forensic community. Compared with father, a more serious Y-interstitial deletion of the son was observed, which suggested that Y-microdeletions in parent would make the Y chromosome more liable to a second mutation in offspring, as a consequence of DNA instability.

Analysis of mitochondrial DNA control region polymorphisms based on denaturing high-performance liquid chromatography

X. Wang[1], Y. Hou[2]

[1]School of Criminal Investigation, Southwest University of Political Science and Law, Chongqing, China

[2]Department of Forensic Genetics, Sichuan University, Chengdu, Sichuan, China

The purpose of this study is to establish a novel method for the detection of mitochondrial DNA (mtDNA) polymorphism based on denaturing high-performance liquid chromatography (DHPLC) and to explore the new mitochondrial DNA polymorphisms in order to improve the discrimination power of mtDNA in forensic DNA typing.

Primers were designed by Primer3 software to amplify two new polymorphism loci in control region of mtDNA. Using a technique of DNA pools and performing the analysis of pair-wise combining samples, the DHPLC methods that base on the most homologous and the retention time from the cartridge were evaluated and optimized. The methods established were employed to investigate the polymorphisms of mtDNA. Then, a series of validation experiments were carried out to evaluate its utilities for forensic purpose.

Finally, The heteroduplexes from the two new loci in mtDNA control region were successfully separated by DHPLC and a protocol suitable for forensic rapid analysis was established with high efficiency and low cost, they were expected to be important methods for forensic mtDNA identification.

Theme 5: X STR polymorphisms

Generic characterisation of an X-STR decaplex on the population of Pichincha (Ecuador)

A. Gaviria[1,2], A.K. Zambrano[1,2], G. Morejon[1,2], V. Aguirre[1,2], M. Vela[1,2], J.J. Builes[3], G. Burgos[1,2]

[1] Laboratorio de Genética Molecular de Cruz Vital S.A. Quito, Ecuador.

[2] Cruz Roja Ecuatoriana, Quito, Ecuador.

[3] Laboratorio GENES Ltda, Medellin, Colombia.

Autosomal STRs genotyping has been used around the world as main option for discrimination of identity in forensic test, biological filiation and paternity cases, because of its high discrimination power. However, in some cases like sexual offenses and complex biological relations, reliability cannot be achieved so, another tools such as SNPs, MiniSTRs, InDels, X-STRs are necessary to conclude those.

This study reports first data of ten STR markers (DXS8378; GATA172D05; DXS6809; DXS7132, GATA31E08, DXS9898, DXS7133, DXS7423, DXS9902, DXS6789) in Ecuadorian population, to achieve these, we selected 100 non related individuals that had signed informed consent (48 women and 52 men) from the population of Pichincha (Ecuador). They were typified using markers previously described by the Spanish and Portuguese ISFG Working Group (GEP-ISFG, 2008) and following their experimental recommendation.

Based on allele distribution, the forensic parameters for each locus were calculated. After bonferroni correction, the population sample under study did not show any deviation from Hardy-Weinberg or linkage disequilibrium. Moreover, the values obtained for power of discrimination were 0.999999987 in females and 0.999994 in males. The Combined Main Exclusion Chance was 0.99999978 in trios and 0.9993 in duos.

INDEL Polymorphisms at the DXS10146 Flanking Region in Four Racial Populations

A. Nagai[1], M. Hara[2], A. Tamura[3], A. Kido[4], Y. Bunai[1]

[1]Department of Legal Medicine, Graduate School of Medicine, Gifu University, Gifu, Japan

[2]Department of Forensic Medicine, Faculty of Medicine, Saitama Medical University, Moroyama, Japan

[3]Department of Legal Medicine, Osaka Medical College, Takatsuki, Japan

[4]Department of Instruction, Nagoya Isen, Nagoya, Japan

Insertion/deletion (INDEL) polymorphisms in the 3' flanking region at the X chromosomal STR DXS10146 were analysed in four racial populations. DNA was obtained from 151 Japanese males, 60 Indonesian males and 90 South African males (32 Caucasians and 58 Africans). All samples were amplified, followed by sequencing. A 17-bp INDEL polymorphism (TTCTTTCTTCTTTCTTT/–) in the 3' flanking region, as described by Sim et al (2010), was observed in all populations. four-bp (TCTT/–) and 7-bp (TTCTTTC/–) INDEL polymorphisms were newly found 14–17 bp and 26–32 bp downstream of the core repeat unit of DXS10146, respectively, in the African population. All deletion events at the INDEL regions were only observed in alleles that had six repeat number polymorphisms at the variable (TTCC) repeat block in the core repeat unit, although 3, 6, and 10–15 repeat number polymorphisms were found in the variable (TTCC) repeat block in this study. None of the insertion events were observed in alleles that had six repeat number polymorphisms at

the variable (TTCC) repeat block in the Japanese and Indonesian populations; however, some insertion events were observed in the alleles that had six repeat number polymorphisms at the variable (TTCC) repeat block in the Caucasian and African populations. Thus, the deletion events in the INDEL polymorphisms in the 3′ flanking region at DXS10146 are strongly associated with the repeat number of the variable (TTCC) repeat block in the core repeat unit.

A multiplex typing system composed of autosomal and X-chromosomal STR markers

C. Tavares[1], S. Loiola[1], I. Pontes[3], D. A. Silva[1,2] and E. F. Carvalho[1,2]

[1] Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[2] Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[3] Instituto de Biologia, Universidade Federal do Amazonas, Manaus, AM, Brazil

Ordinary paternity cases are successfully conclusive by applying a commercial multiplex system, as the Identifiler or Powerplex PP18, for instance, in the laboratory routine. However, the LR becomes smaller by a factor of 102 to 104 when the occurrence of one/two STR loci paternity putative mutation(s) and a larger number of loci should be genotyped. Moreover, one commercial kit is not enough to solve the majority of cases in which relatives of an absent alleged father take his place in the genetic investigation. As a supplemental tool for those kind of cases, a human DNA multiplex typing system (AX9) comprising seven autosomal (D1S1656, D12S391, D16S539, Penta D, Penta E, D3S18773 and GH15) and two X chromosomal (DXS7133 and DXS10074) STRs markers has been developed in our laboratory. The D3S18773 and GH15 loci have not been already described and statistical parameters are presented for them. The X-chromosome markers are particularly important for comparisons among an alleged father, or his mother, and a female child as well as for cases of maternity investigation. The usefulness of the AX9 multiplex as an additional genotyping system for complex kinship tests was tested. On one hand, the results have shown a very important increase on the LR ratios or, on the other hand, a larger number of inconsistent loci.

Keywords: new STR loci, multiplex, autosomes, X-chromosome, kinship

Financial support: Justice Court and Public Prosecuting DNA Program, Faperj, CNPq and CAPES

The polymorphisms of 12 X-STR loci in six ethnic populations in China

H.Y. Sun[1], Z. Ren[1], X.P. Zeng[1]

[1] Department of Forensic Genetics, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

Introduction and aims X chromosomal STR (X-STR) is in sex-linked inheritance and has its advantage in deficiency paternity cases and forensic caseworks. The aim of the study is to explore the genetic polymorphisms of 12 X-STR loci for Guangdong Han population and other five ethnic populations (Tibetan, Mongolian, Korean, Uighur and Hui) whose population size is over 1 million in China.

Materials and methods 1292 samples from unrelated individuals of these 6 ethnic populations were amplified with Investigator™ Argus X-12 multiplex PCR system. PCR products were separated by ABI 3500XL Genetic Analyzer and genotyped by GeneMapper® ID-X software.

Results and discussion 238 alleles were observed totally, which included 66 off-ladder alleles. All the loci showed no difference in sex-related allele frequency. The combined discrimination power was 0.999999999 in males (CDPM) and 0.999999999 in females (CDPF) for the whole samples, and the combined mean exclusion chance was ranged from 0.999998336~0.9999999257 in duo cases and 0.999999987~0.999999999 in trio cases in 6 populations. All of the 12 loci were in accordance with Hardy-Weinberg equilibrium after Bonferroni's correction. Linkage disequilibrium was observed for DXS10103-DXS10101 pair in 6 populations. Significant differences between studied populations and other populations were observed except Han-Hui and Mongolian-Hui population-pair. The phylogenetic tree consisted of two main branches for these 6 populations, which was consistent with the geographic and historic distributions.

This study was supported by the National Natural Science Foundation of China (81273347) and the Fundamental Research Funds for the Central Universities (09YKPY78).

Genetic data of 10 X-STRs in a population sample from Lima, Perú

C. Barletta[1], J.J. Builes[2],3, M.L. Bravo[2], D. Aguirre[2], J. Descailleaux[1], L. Gusmão[4]

[1] Laboratorio de Genética Humana, Universidad Nacional Mayor de San Marcos, Lima, Perú.

[2] GENES Ltda, Medellín, Colombia.

[3] Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

[4] IPATIMUP, Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal.

The Chromosome X-STRs were recently recognized as useful tools in forensic kinship testing, mainly in solving of complex cases. The aim of this study was describe the polymorphism of 10 X-STR loci in a Peruvian population sample, and evaluate their efficiency in forensic practice and paternity testing.

A set of 282 samples, 141 males and 141 females, obtained from unrelated donors of the Lima population were analyzed using 10 X-STRs -DXS6789, DXS9902, DXS7132, GATA31E08, DXS7133, DXS9898, DXS8378, DXS6809, DXS7423 and GATA172D05- through PCR multiplex. The allele frequencies and mutation rates of the ten loci were investigated, and the comparison of allele frequency distribution with other populations (Latin American, Iberian and African) was performed. Statistical analysis was performed using PowerStats and Arlequin software**.**

The female population showed no deviation from Hardy-Weinberg equilibrium at all loci analyzed. DXS7132 was the most polymorphic marker (PIC = 0.7615) with a probability of discrimination in males and females of 0.7913 and 0.9267, respectively. The combined use of these markers produced high values of mean exclusion chance in trios involving daughters and in father/daughter duos of 0.9999752595 and 0.999221451, respectively, demonstrating the usefulness of this set of markers in forensic and kinship analysis. Four mutations were observed in 138 trios father/mother/daughter studied using the same set of X-STRs. Pair-wise comparisons of allele frequencies distribution showed significant differences for most loci among different populations studied. The study of these X-STRs contributed to the establishment of a database for X chromosome markers in Lima-Perú population.

Using autosomic STR and X-STR markers to solve a complex case of biological relationship of a daughter abandoned by her mother and with deceased alleged father.

M.L.J Bravo[1], A Manrique[1,2], D.P. Aguirre[1], L Mendoza[1,3], J.J. Builes[1,2]

[1] *Laboratorio GENES Ltda, Medellín, Colombia.*

[2] *Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.*

[3] *Universidad Pontificia Bolivariana, Medellín, Colombia*

Colombia has suffered a civil war for over 50 years, which has left, a large number of dead and missing people, people forced to displacement from the fields to the cities, creating many complex cases of biological relationships (BR) to investigate. This research was conducted with the purpose of supply a conclusive diagnosis for Biological Father-Daughter Relationship (BFDR) of a girl who was abandoned by her mother and her alleged father is dead.

For this research, we only had the alleged father's mother and alleged paternal uncle. We used 15 autosomal STR loci (PowerPlex ® 16 Bio, Promega Co.) and the Decaplex X-STRs (GHEP-ISFG) for the genetic profiles of the people studied in this BR. Genes Ltda participates annually in the interlaboratory quality controls of the Spanish and Portuguese Speaking Working Group of ISFG (GHEP-ISFG) and with the Colombian Group of Human Identification and Forensic Genetics (GCIHyGF).

With the combination of STR autosomal and X-STRs in this research we reach a RB Index equals 7606420.2169 and a chance of BR (WBR) of 0.99999987, fulfilling the required values for the legal validity specified in Article 2 of the Law 721 of 2001 in Colombia. Also shows the informational efficiency of the X-chromosome STR loci in complex investigations of biological relationships.

Estimating relatedness with no prior specification of any genealogy: The role of the X-chromosome

N. Pinto[1], L. Gusmão[1,2], T. Egeland[3], A. Amorim[1,4]

[1] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal*

[2] *DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro - RJ, Brazil*

[3] *Norwegian University of Life Sciences, Department of Chemistry, Biotechnology and Food Science, Norway*

[4] *FCUP, Faculty of Sciences of the University of Porto, Portugal*

Inferential studies on genetic relatedness fall into two broad approaches: (a.) the genetic types of the individuals are used to compare the likelihood of their relatedness through specific genealogies; or (b.) the genetic information is evaluated to infer the degree (or level) of relatedness between the individuals without specifying any pedigree. Approach (a.) is used to compute statistics when the alternative pedigrees and/or samples are few (such as in the case of a standard paternity test, for example), while approach (b.) is more general, and useful to bring into light cryptic, undisclosed or unspecified kinships (suited, for example, to seek for related individuals at mass disasters comparing recovered and reference samples). From a theoretical point of view, framework (a.) is based on the detailed pedigrees description given by the complete set of identical-by-descent probabilities, while (b.) aims, in most cases, at the estimation of the coancestry coefficient θ, which is generally assumed as a coarser measure of genetic relatedness since it clusters pedigrees, such as full-siblings and parent-child, which are distinguishable through framework (a.). In this work, under framework (b.), we show that extending the standard estimation of the autosomal coancestry to X-chromosomal transmission can be a powerful approach to bring into light distant genealogies and to discern specific pedigrees, mainly if combined with the estimation of the autosomal counterpart. Indeed, for the latter purpose, the combined statistics for autosomes and X-chromosomal markers can reveal the same power as that obtained with the, so far, unbeatable framework (a.).

Typing of 67 SNP loci on X chromosome by multiplex amplification followed by MALDI-TOF MS

L. Li[1], Y. Liu[1], Y. Lin[1], C. Li[1], S. Zhang[1], S. Zhao[1]

[1] *Shanghai Key Laboratory of Forensic Science; IFS, Ministry of Justice, China*

Objective: To screen out a panel of X-SNP loci that is informative in Chinese Han population and highly valuable in forensic identification.

Method: 67 candidate SNP loci located X chromosome were selected according to the information on NCBI and HapMap homepages. Genomic DNA samples extracted from 295 unrelated Chinese Han individuals were analyzed through multiplex amplification followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and allele frequencies of 67 X-SNP loci were calculated. In view of the population data and situation of linkage disequilibrium, X-SNP markers promising in forensic identification were screened out

Results: Population data of 67 X-SNPs were obtained except rs12849634, no deviations from Hardy-Weinberg equilibrium could be found. Two out of the other 66 X-SNP loci (i.e.rs1229078, rs1544545) were found to be low informative (MAF<0.3).Tight LD were observed at six groups ;rs5986750 vs rs5986751; rs183277 vs rs7060326; rs6611148 vs rs4826623; rs5923750 vs rs1166756; rs6620798 vs rs5917032; rs2519557 vs rs6649211 and slight LD were found at two groups (rs4276834 vs rs5928614; rs5968332 vs rs6418330). 52 X-SNP loci showing independent inheritance and high polymorphisms were finally selected out. The markers were promising in forensic identification. The accumulative exclusion probabilities (CPE) in trio cases and duo case were 0.99999999996 and 0.9999995 respectively. The combined discrimination power (CDP) in female population and male populations was 0.9999999999999999999 and 0.99999999999999931 respectively.

Conclusions: A panel of 52 informative X-SNPs showing independent inheritance was screened out. The markers meet the needs of individual identification and relationship testing about special disputed cases.

An assessment of the genetic structure of Indian population through typing of X chromosomal SNPs of forensic interest

S. Mukerjee[1], M. Mukherjee[1], T. Ghosh[1], D. Kalpana[1], and A. K. Sharma[1]

[1] *Central Forensic Science Laboratory, Directorate of Forensic Science Services, Ministry of Home Affairs, Govt. of India, Kolkata- 700014, West Bengal, India*

Since all daughters of the same biological father would share at every X chromosomal locus an allele identical by descent, hence typing of X chromosomal SNPs might be more informative than autosomal SNPs in deficiency paternity cases. Haplotype can also be easily determined by studying X chromosomal SNPs in male individuals since they possess a single copy of this sex chromosome.

Indian populations are classified into four major linguistic families, namely, Indo-European (IE), Dravidian (DR), Tibeto-Burman (TB) and Austro Asiatic (AA). In the present study, we examine the genetic variation among ten endogamous Indian populations using X SNPs. The studied populations are distributed over six geographical regions and are socially stratified into castes and tribes.

Twenty four X chromosomal SNPs were amplified according to Tomas *et al.* (2008) in unrelated male individuals from 10 populations of India. The amplicons were electrophoresed on an ABI PRISM® 3100 Genetic Analyzer and data was analyzed with GeneMapper® ID Software v3.2.

Allele frequencies were estimated for the 24 X-SNPs. Arlequin programme v3.1 was used to compute the haplotype frequencies, gene diversity, *p* values of exact test for linkage disequilibrium, population pair-wise genetic distances (F_{ST}) and analysis of molecular variance (AMOVA) among the studied populations for the X-SNPs. The Neighbour-Joining (NJ) phylogenetic tree for the X-SNPs was drawn and a MDS plot was created.

For the 24 X SNP markers, minor allele frequency (MAF) ranging from 18.3-81.7 %. A total of 600 different haplotypes were observed, with no evidence of haplotype sharing within and among the populations. The F_{ST} values ranged from 0.01137-0.09268, indicative of the genetic relatedness or distance between populations of the diverse linguistic families, geographic locations and social strata. The tribal populations of IE, DR, TB and AA groups were observed to be significantly distant from the caste populations. AMOVA analyses indicated that the within populations variation accounts for about 95-96% of the total diversity observed among Indian populations, while about 3.6% of the variation is a result of the interpopulation diversity. These results illustrate that the diverse population origins, histories and geographical localizations and barriers amount to the extensive genetic heterogeneity among the extant Indian populations.

The results of the MDS plots were in accordance with the confounding influences of language and geography. IE and DR families displayed little variance which might be due to the prevalent invasion theory of nomadic IE speakers into the pre-existing society of DR speakers, with the resultant introduction of the hierarchical caste system into the society.

Characterization of X chromosomal short tandem repeat markers for forensic use: a project overview

T.M. Diegoli[1,2,3], A. Linacre[3], and M.D. Coble[4]

[1]*Armed Forces DNA Identification Laboratory, Armed Forces Medical Examiner System, Dover Air Force Base, DE, USA*

[2]*American Registry of Pathology, Camden, DE, USA*

[3]*Flinders University, Adelaide, SA, Australia*

[4]*National Institute of Standards and Technology, Gaithersburg, MD, USA*

The use of X chromosomal short tandem repeat (STR) markers has been greatly increasing in the forensic setting. The marker system offers the potential to provide information in addition to what is obtained from autosomal STR systems currently used at crime laboratories and in the courtroom. In certain scenarios, markers on the X chromosome may be the only means of obtaining this information. In-depth characterization of the marker system is the first step in maximizing the power of this additional tool in the forensic arsenal.

Using guidelines set forth within the 1991 report of the International Society for Forensic Genetics (ISFG) relating to the use of DNA polymorphisms, all aspects of the feasibility of routine X chromosomal STR use were evaluated. Two mini-X chromosomal STR multiplexes capable of amplifying 15 total markers were developed and utilized to determine allele nomenclature, allele/genotype frequencies, mutation rates, and linkage between markers. Additionally, a concordance study between these multiplexes and a commercially available kit was performed. Lastly, applications of the marker system in specific kinship situations and mixture analysis were explored. Here, the authors present a comprehensive overview of each portion of this extensive developmental validation study.

Portions of this work were supported by U.S. National Institute of Justice grant award 2011-DN-BX-K401.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense, the United States Department of the Army, or the National Institute of Justice.

Theme 6: Ancestrally informative DNA markers in forensic science

Genome-wide ancestry *versus* ancestry inferred from AIMs panels

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

[1] *Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain*

[2] *Pediatric Emergency and Critical Care Division, Department of Pediatrics, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Galicia, Spain*

[3] *Grupo de Investigación en Genética, Vacunas, Infecciones y Pediatría (GENVIP), Hospital Clínico Universitario and Universidade de Santiago de Compostela (USC), Galicia, Spain*

Quite often, new panels of ancestry informative markers (AIMs) aimed to measure biogeographical ancestry of individual profiles or population samples are developed by forensic and population geneticists. These panels can be particularly useful for police investigation when assessed on evidentiary samples but also in anthropological and clinical studies. The efficiency of these panels is generally proved empirically by way of comparing samples of already 'known' ancestry (e.g. self-declare ancestry) with the ancestry inferred using these panels. Using SNP data from HapMap (http://hapmap.ncbi.nlm.nih.gov) we first carried out a genome-wide simulation-based

study aimed to measure the effect of varying the number of SNPs when measuring genome ancestry. Genome-wide ancestry was then compared to ancestry measured using already pre-designed AIMs panels. The data indicated that continental ancestry measured using sets of 1,000 SNPs randomly selected from the genome approaches much better to whole genomic ancestry than ancestry estimated using pre-designed panel. Inferences of ancestry using 100 randomly selected SNPs are exposed to higher errors but could in some cases perform better than some pre-designed AIM panel, including those panels containing >100 AIMs. In general, the results indicate that ancestry inferred from AIM panels and assessed on population samples (average population ancestry) approaches reasonably well to whole genomic ancestry although some continental ancestry values could be over/under-estimated depending on the AIM set; larger errors are however expected for those panels containing lower number of SNPs. When assessing ancestry in evidentiary samples (individual profiles) errors on ancestry estimates could be unpredictable and relevant in forensic casework.

The research leading to these results has received funding from the People Program (Marie Curie Actions) of the European Union's Seventh Framework Program FP7/2007-2013/ under REA grant agreement n° 290344, and the grants from the "Ministerio de Ciencia e Innovación" (SAF2008-02971) from the Plan Galego IDT, Xunta de Galicia (EM 2012/045) given to AS.

Delineating genetic structure of the Mayan from Guatemala by uniparental and ancestry informative markers

J. Söchtig[1], V. Álvarez-Iglesias[1], M. Gelabert-Besada[1], A. Mosquera-Miguel[1], Á. Carracedo[1], M. V. Lareu[1], A. Salas[1]

[1] Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, 15872, Galicia, Spain

In Guatemala, Native indigenous people of Maya descent comprise about 40% of the population. The main aim of this study is to analyze the genetic variability of the Maya population from Guatemala by means of uniparental and ancestry informative markers (AIMs). A total of 121 DNA samples were recruited comprising 28 Guatemalan 'mestizo' inhabitants (supposed to be admixed between Mayans and Spaniards), and 93 Guatemalan Mayan individuals comprising 70 Q'eqchi', 15 Pokom, 3 Pokomchí, 2 Achí, 2 K`akchikel, and 1 K'iche. We genotyped a set of Y chromosome biallelic polymorphisms (Y-SNPs) in Guatemalan males in order to allocate individual profiles into main continental ancestries. A total of 110 samples were genotyped for the mitochondrial DNA (mtDNA) entire control region and haplotypes were classified into haplogroups. Furthermore, AIMs were assessed in order to infer biogeographic ancestry. Results of uniparental loci showed that the Guatemalan population samples are mainly composed by Native American haplogroups with little presence of sub-Saharan and/or European lineages. Analysis of entire genomes of few Guatemalan mtDNA lineages is under process given their particular features when contrasted with the known Native American phylogeny. AIMs also indicate the predominant Native American nature of Guatemalans. The data generated in the present study represents one of the very few genetic studies carried out in Guatemalans to date, and the ethnic groups represented by this sampling are analyzed here for the first time. The data is therefore of particular interested from the population and forensic genetic point of view.

JS and VAI were supported by grants from the German FAZIT-STIFTUNG and EUROFORGEN, respectively. The research leading to these results has received funding from the People Program (Marie Curie Actions) of the European Union's Seventh Framework Program FP7/2007-2013/ under REA grant agreement n° 290344, and the grants from the "Ministerio de Ciencia e Innovación" (SAF2008-02971) from the Plan Galego IDT, Xunta de Galicia (EM 2012/045) given to AS.

The EUROFORGEN forensic ancestry marker exercise

C. Phillips[1], C. Santos[1], M. Fondevila[1], W. Parson[2], D. Ballard[3], C. Børsting[4], W. Branicki[5], M. Eduardoff[2], T. Gross[6], P. Kohler[7] and the EUROFORGEN-NoE Consortium

[1] Forensic Genetics Unit, University of Santiago de Compostela, Spain.

[2] Institute of Legal Medicine, Innsbruck Medical University, Austria.

[3] Kings College Forensic Science, Life Sciences, Kings College, London, UK.

[4] Department of Forensic Medicine, University of Copenhagen, Denmark.

[5] Department of Genetics and Evolution, Jagiellonian University, Krakow, Poland.

[6] Institute of Legal Medicine, University of Cologne, Germany.

[7] Norwegian Institute of Public Health, Oslo, Norway.

The EUROFORGEN forensic ancestry marker exercise compared two established systems for forensic ancestry analysis: a SNaPshot test of 34 ancestry-informative single nucleotide polymorphisms (AIM-SNPs) and a dye-labeled direct PCR test of 46 AIM-Indels (Fondevila et al. 2013, Pereira et al. 2012 respectively). Both tests have recognized advantages and disadvantages, so the exercise was designed to highlight these for participating laboratories that, in the main, were applying one or both genotyping techniques for the first time. Participants typed NIST SRM 2391c standard control DNA samples plus four population-representative donors in order to cover all five major population groups, as well as analyzing one example of mixed DNA (SRM 2391c samples A+C=D). Participants then genotyped their choice of in-house study populations to help expand the scope of reported data in the online *SPSmart* frequency browser, with forensic AIM-Indel pages initiated from data collected by the exercise.

We report the exercise findings, focusing on: ease of use of each genotyping approach; interpretation of large-scale multiplex profiles when peak patterns cannot be fully equilibrated across different laboratories; mixture detection; use of the *Snipper* online forensic classifier to assess admixed individuals showing co-ancestry and; handling large sets of SNP and Indel profiles with the *Snipper* tool.

M. Fondevila et al. (2013) Revision of the SNP*for*ID 34-plex forensic ancestry test: Assay enhancements, standard reference sample genotypes and extended population studies, Forensic Sci. Int. Genet. 7: 63–74.

R. Pereira et al. (2012) Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing. PLoS One 7(1): e29684.

Acknowledgements: This work was funded by the EUROFORGEN-NoE (Grant Agreement No. 285487).

Comparative Analysis of Two InDel-based Ancestry Informative Multiplex PCR Typing Kits

D. Zaumsegel[1], R. Pereira[2], M. A. Rothschild[1], C Phillips[3], L. Gusmão[2,4], P. M. Schneider[1] and the EUROFORGEN-NoE consortium

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

[2] IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal

[3] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain

[4] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro - RJ, Brazil

Insertion/deletion polymorphisms have recently received increased interest as a novel marker class in forensic genetics combining the advantageous genetic properties of single nucleotide polymorphisms (i.e., low mutation rate, genetic stability, and short amplicon size) with the technical advantage of short tandem repeat markers (simple and rapid detection by fluorescence-labelled PCR followed by capillary electrophoresis). Several panels utilising indel markers for different objectives in forensic genetics such as identification of individuals, paternity testing or inference of biogeographic ancestry have been published in recent literature.

For the study presented here, two recently published indel marker sets for the inference of biogeographic ancestry (46plex: Pereira et al., PLoS ONE 7(1), 2012, e29684; 21plex: Zaumsegel et al., FSI Genetics 7(2), 2013, 305) have been applied on a subset of the CEPH Human Genome Diversity Panel as well as some study populations of our own in order to compare the effectiveness of the marker sets in differentiating the populations on a continental level. Preliminary data suggests both multiplexes are not able to clearly discriminate European from Middle East populations. Therefore, the main focus of the comparison are South, South East, and East Asian populations as well as Oceanian and the Amerindian populations not yet subjected to typing with the 21plex. The results will provide information about the performance of both kits for these populations, and whether a combined analysis of 67 InDels can increase the prediction efficiency.

Ancestry inference in a multicultural population

D.J. Ballard[1], A. Revoir[1], C. Phillips[2], D. Syndercombe Court[1]

[1] Department of Forensic and Analytical Science, King's College London, UK

[2] Forensic Genetics Unit, University of Santiago de Compostela, Spain

The British population is made up of three main ethnic groups: Caucasian/White British, Black British and South Asian. The history of Britain is littered with a series of invasion and colonisation events, potentially resulting in a variety of different genetic influences shaping the native population. More recent immigration trends have led to 6.5 million people within the UK describing themselves as belonging to an ethnic minority, comprising a significant or majority group in some urban areas. The aim of this research is therefore to characterise the major British population groups for a series of genetic markers, and ultimately developing a robust population-of-origin classification system for a forensic DNA sample of unknown origin.

To this end, an established set of 34 single nucleotide polymorphisms (SNPs) were genotyped along with a small supplementary panel of SNPs specifically designed to increase South Asian differentiation. Individuals resident within the UK from the three major populations, along with an East Asian (exclusively British Chinese) subset were genotyped and population-of-origin classification performed using a modification of the Snipper portal (http://mathgene.usc.es/snipper/). Classification could be achieved in 91% of cases (with the remaining 9% classified as 'unknown') and of those individuals classified there was 100% concordance between prediction and self-declared ancestry. A 50 sample blind trial was conducted containing a mixture of samples from around the globe that demonstrated the robustness of this approach.

Increased resolution of U.S. population structure from the application of an Ancestry Informative InDel genotyping assay.

M. Fondevila[1,2], C.Santos[1], C. Phillips[1], J.M. Butler[2], Á. Carracedo[1], M.V. Lareu[1], P.M. Vallone[2].

[1]Instituto de Ciencias Forenses "Luis Concheiro", Unidade de Xenética Forense,Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

[2]National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, USA

Admixture is increasingly common in many human populations. The additional level of complexity in the patterns of genetic variation found in admixed populations cancomplicateancestry inferencesmade from casework DNA if inappropriate reference allele frequencies are applied. Inrecent years, several biallelic marker assays for the prediction of biogeographical ancestry have been published and these have achieved success in forensic applications. Ancestry analysiscan increase our knowledge of the complexity of admixed human groups and help build more comprehensive population reference data leading to more precise prediction of biogeographical origin when individuals come from complex population backgrounds.

We have applied an InDel assay designed for prediction of biogeographical ancestry (Pereira et al., 2012) to a set of representative U.S. population samples from NIST. This set represents a suitablereference panel for our purposes since the samples are distributed among four self-declared ancestry groupswith different degrees of historical admixture. We have also made use of previously obtained SNP data for those same populations with the 34-plex AIM-SNP assay (Phillips et al. 2007, Fondevila et al. 2013) in order to increase the power of our analyses and improve insight into U.S. population admixture and substructure patterns.

Allele frequencies for the 46 InDels of the assay have been generated with a significant sample number for each population group. Our data contributes to improved characterization of admixed populations and their analysis in forensic applications through biallelic marker genotyping.

R. Pereira et al., (2012) Straightforward Inference of Ancestry and Admixture Proportions through Ancestry-Informative Insertion Deletion Multiplexing. PLoS One 7(1): e29684

C. Phillips, et al., (2007) Inferring ancestral origin using a single multiplex assay of ancestry-informative marker SNPs, Forensic Sci. Int. Genet. 1: 273–280.

M. Fondevila et al., (2013) Revision of the SNPforID 34-plex forensic ancestry test: Assay enhancements, standard reference sample genotypes and extended population studies, Forensic Sci. Int. Genet. 7: 63–74.

Theme 7: Single nucleotide polymorphisms in forensic science

On how many SNPs are needed to make reliable classifications

A. Gómez-Tato[1], J.A. Álvarez-Dios[1], M.A. Casares de Cal[1]

[1] *Faculty of Mathematics, University of Santiago de Compostela, Spain.*

The number of independent independent single nucleotide polymorphisms (SNPs) needed to reliably classify an individual as belonging to a population is related to their cumulative informativeness and the number of populations compared. By using computer simulations, we have studied the relationship between the amount of informativeness needed to reach a good success ratio, and a fixed number of populations, when a Naïve Bayesian system is used to make a classification, for instance, as applied with the *Snipper* App Suite (http://mathgene.usc.es/snipper/) —an online forensic profile classifier applicable to a range of forensic classification analyses—. We have also explored the possibility to weaken the hypothesis of marker independence assumed when applying the Bayesian classifier.

High resolution melting (HRM) analysis of forensically informative SNPs

B. Mehta[1], R. Daniel[2], D. McNevin[1]

[1] *National Centre for Forensic Studies, University of Canberra, ACT, Australia*

[2] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Victoria, Australia*

The development of the SNPforID 34-plex, IrisPlex and HIrisPlex assays has demonstrated the applicability of the SNaPshot® assay for SNP genotyping in forensic DNA analysis. However, the SNaPshot® assay is a multi-step process with an associated post-PCR contamination risk. An alternative is the single tube high resolution melting (HRM) temperature real-time PCR method. HRM eliminates the post-PCR requirement of SNaPshot®. Eight individual DNA samples were genotyped at the six IrisPlex SNP loci using both the IrisPlex published primer set and a set of custom designed HRM primers. The performance of both sets of primers was examined using MeltDoctor™ (Life Technologies®) and SensiFast™ (Bioline®) HRM mastermixes on the ViiA™ 7 real time PCR platform over a range of DNA template amounts. The resultant genotypes were compared with those derived from SNaPshot®. HRM offers a viable, faster and flexible alternative to SNaPshot® for small numbers of SNP loci without the associated contamination risk from post-PCR processes.

Development of 30 InDel markers typing system and genetic analysis in five different Chinese populations

S.M. Zhao[1], S.H. Zhang[1], K Sun, C.T. Li[1]*

[1] *Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, P.R. China, Shanghai, P.R. China*

Insertion/Deletion (InDel) markers belong to an important class of molecular markers with the advantages of both SNPs and STRs, such as lower mutation rate, improved application in the analysis of degraded samples, applicable to the genotyping platform of STRs. 30 InDel markers were selected with the Human Genome Browser in Galaxy system and dbSNP database according to designed criteria. A new multiplex polymerase chain reaction (PCR) system which can simultaneously analyze 30 InDel markers was then developed and validated. The samples of 419 unrelated individuals from five different populations of Han, Hui, Uighur, Mongolian and Tibetan in P.R. China were successfully analyzed. Data were performed with softwares of PowerStats V12 and GenAlEx V6.3. The results for Hardy-Weinberg equilibrium tests and linkage disequilibrium analysis showed that allele frequency distributions of the 30 InDel markers had meet the genetic equilibrium in all of the five populations and the InDel markers on a same chromosome didn't generate any linkage block. Analysis of molecular variance (AMOVA) indicated that genetic variation among the 5 studied populations represent only 4.00% of the total genetic diversity. We observed the cumulative power of discrimination (CPD) for each studied population was 0.99999999999841 in Han population, 0.99999999999690 in Hui population, 0.99999999999709 in Uighur population, 0.99999999999772 in Mongolian population and 0.99999999999854 in Tibetan population. Our work indicates that this multiplex system is useful for forensic analysis for the five populations in China. Research supported by the Ministry of Science and Technology of the People's Republic of China (2012BAK16B01).

Application of Jacobs syndrome using Quantifiler® Duo DNA Quantification Kit

C.H. Kim[1], S.O. Moon[1], M.J. Kim[1], K.D. Kwak[1], J.Y. Kim[1], J.Y. Jung[1], D.H. Choi[1], K.W. Park[1].

[1] *Forensic DNA Center, National Forensic Service, Seoul, South Korea*

Jacobs syndrome (XYY syndrome) is made an aneuploidy of the sex chromosomes when a human male receives an extra Y-chromosome. The person is mostly normal male however he may suffer from excessive male hormones, such as excessive acne, taller than normal and aggressive behaviors. The discovery of this phenomenon was confirmed mainly through karyotyping of chromosomes using Fluorescence In Situ Hybridization (FISH), but we studied using the Quantifiler® Duo DNA Quantification Kit. This kit enables to simultaneously obtain a quantitative and qualitative assessment of total human and human male DNA. Also in forensic science we has been used this kit to quantify the amount of male DNA from mixed samples.

As a result of real-time PCR using this kit, we confirmed that the amount of male DNA who has Jacobs syndrome is approximately twice that of normal male.

A human tri-allelic SNP catalog for forensic use

C. Phillips[1], M. Fondevila[1], J. Amigo[2], M. de la Puente Vila[1], M. Torres[2], I. Quintela[2], Á. Carracedo[2], M.V. Lareu[1]

[1] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain

[2] Grupo de Medicina Xenómica, University of Santiago de Compostela, Spain

With the growing interest in Next-Gen sequencing technologies applicable to forensic SNP typing, tri-allelic SNPs have resurfaced as potentially important aids to the detection of mixed DNA from the sequence data that such technologies produce. In situations where allele call imbalances, particularly at low sequence coverage, may be indistinguishable from mixture patterns, the presence of a third SNP allele can help reveal true mixed-source DNA commonly encountered in forensic analysis.

We have validated 29 tri-allelic SNPs with the 944-sample HGDP-CEPH global population panel, with all loci showing three alleles at relatively high frequencies in some, but usually not all, populations. In addition, we screened the publicly released *Complete Genomics* data of 69 full genome sequences to detect the presence of three alleles at 400 tri-allelic sites listed as the most polymorphic by dbSNP. The catalog of tri-allelic SNPs compiled can help build panels for mixture detection in Next-Gen sequencing approaches, but many of the SNPs also have considerable potential as ancestry informative markers since their allele frequencies exhibit larger skews in population distributions than the majority of binary SNPs.

High-confidence detection of distant relationships using high-density SNP genotypes obtained from small amounts of degraded DNA

D.J. Witherspoon[1], J. Tackney[1], T. Parsons[2], L.B. Jorde[1]

[1] University of Utah, Salt Lake City, Utah, USA

[2] International Commission for Missing Persons, Sarajevo, Bosnia and Herzegovina

Introduction: The ability to identify individuals by comparing forensic DNA to samples from potential relatives is of central importance to forensics. Current methods require either direct reference samples or samples from multiple immediate relatives to permit high-confidence identification. ERSA (Estimation of Recent Shared Ancestry) is a method that uses high-density SNP genotype data to detect distant relationships (up to 3rd cousins). However, generating such data from small quantities of fragmented DNA and detecting distant relationships using more limited and error-prone data is not currently possible.

Methods: 24 DNA samples were prepared, ranging from high-quantity (100 ng), high-quality, unfragmented samples to low-quantity (0.1 ng), partially degraded and mechanically fragmented ones (<300 bp). Samples were repaired, whole-genome amplified and genotyped at >700,000 SNP loci with Illumina Infinium HumanOmniExpress-FFPE BeadChips.

Results: Genotyping success rates of >95% were achieved with ≥5 ng of fragmented DNA. Products amplified from 1 ng fragmented DNA matched control sample genotypes at 88% of loci. This allows high confidence detection of relationships as distant as 3rd cousins. Fewer SNPs are retrieved from 100 pg DNA, but even very poorly performing samples (<<50% genotyped) allow strong inferences of first-cousin relationships.

This research was supported by NIJ award 2012-DN-BX-K037 and Utah TCIP award 121793.

Human DNA identification involving the analysis of haplotypes containing multiple single nucleotide polymorphisms

H. Nakahara[1], K. Fujii[1], T. Kitayama[1], Y. Iwashima[1], K. Sekiguchi[1]

[1] National Research Institute of Police Science, Kashiwanoha 6-3-1, Kashiwa, Chiba 277-0882, Japan

Single nucleotide polymorphism (SNP) typing has a low discriminatory power because of the bi-allelic nature of SNPs, so requires large numbers of loci to be analyzed for the forensic identification of human DNA. Other disadvantages of the technique include difficulties in estimating potential contamination. To overcome these issues, we analyzed haplotype loci including multiple SNPs to generate multiple haplotypes through a combination of SNP alleles. Initially, 27 regions were picked out from the Japanese SNP database (http://snp.ims.u-tokyo.ac.jp) according to our criteria: (1) more than three SNP loci within 100 bp; (2) location within an intron or outside a gene; and (3) an SNP allele frequency > 40%. PCR amplification and high resolution melting curve analysis were carried out for all selected regions to estimate haplotype variation. Haplotype frequencies were observed by direct sequencing of over 100 individuals, and the following seven regions containing the indicated three SNP loci were estimated to have more than three haplotypes: 1q25 (SNPs rs#543912, 338565, 338564), 1q42.2 (SNPs rs#2296796, 2296797, 2296798), 3p24 (SNPs rs#2293140, 2293141, 2293142), 10p13 (SNPs rs#2277214, 2277215, 2277216), 11p15.1 (SNPs rs#2074310, 2074311, 2074312), 14q12-q13 (SNPs rs#2281629, 2281630, 2281631), 20q12 (SNPs rs#2070640, 2070639, 2070638). Such haplotyping will increase the power of individual identification and enable DNA sample mixtures to be estimated by detecting more than three haplotypes. Furthermore, the region of analysis will be shorter than that of standard short tandem repeat analysis and offer advantages for SNP typing of fragmented DNA samples.

Potential forensic application of closely linked autosomal STR haplotype in complex kinship testing

J.P. Du[1], P.Y. Chen[1], Y. Huang[1], J. Zhang[1], Y.B. Li[1,]

[1]Department of Forensic Genetics, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, P.R.China.

It has been noticed that the most commonly used commercial STR kits and mtDNA may not be able to solve some special kinship cases, such as alleged aunt, uncle, niece, nephew or half-siblings. Due to its unique hereditary pattern, the haplotype of genetic markers could be a solution of these questioned family relationships. In this study, we investigated the genetic features of an autosomal STR cluster by employing confirmed family samples. To evaluate the forensic practical value of autosomal STR haplotype, 5 closely linked STR loci, D1S212-D1S2138-D1S3460-D1S164-D1S518, which were arranged in about 2cM region (from 186.29cM to 188.02cM; 1cM represents 1% average recombination between two loci) on chromosome one, were selected to compose haplotype. Genotyping of 60 samples from 8 trios (father-mother-children), 8 duos (father or mother-children), and 4 three-generation pedigrees were performed using PAGE. Haplotypes were identified in the child by determining alleles for all 5 loci transmitted from each parent. Total 73 haplotypes were detected in all samples and 34 haplotypes were observed to be passed down as a whole and was corresponding with the inherited characteristics of haplotype. In all family members, 34 unrelated individuals contributed 65 haplotypes, of which 62 haplotypes appeared only once and the rest 3 haplotypes appeared twice. No recombination was observed in 4 three-generation pedigrees. In conclusion, the haplotype consisting of 5 closely linked autosomal STRs could pass down steadily as a whole. The family specificity of most haplotypes may provide a unique advantage in forensic complex kinship testing.

Population genetic data of 38 autosomal indels in San Basilio de Palenque, the first free town in America

B Martinez[1], J.J. Builes[2,3], A Gaviria[4], G Burgos[4], A Manrique[2,3], D.P. Aguirre[2], L Mendoza[2,5], M.L.J Bravo[2], R Pereira[6], L Gusmão[6], J Marrugo[1]

[1] *Instituto de Investigaciones Inmunológicas, Universidad de Cartagena, Cartagena, Colombia.*

[2] *Laboratorio GENES Ltda, Medellín, Colombia.*

[3] *Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.*

[4] *Laboratorio de Genética Molecular, Cruz Roja Ecuatoriana, Quito, Ecuador*

[5] *Universidad Pontificia Bolivariana, Medellín, Colombia*

[6] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal*

San Basilio de Palenque is located near Cartagena de Indias - Colombia, and was founded by escaped slaves mainly from Cartagena in the sixteenth century. For its history, traditions, ethnicities, culture and language, Palenque has been declared as Intangible Cultural Heritage of Humanity by UNESCO. Palenque is also considered the first free town in America. The aim of the present work was to characterize a San Basilio de Palenque population sample using 38 non-coding bi-allelic autosomal indels and evaluate their utility in forensic.

Samples of 114 unrelated individuals from the San Basilio de Palenque were taken. The DNA was isolated by salting-out and quantified by NanoDrop. It was used the 38 indel-plex developed by Pereira et al 2009. Separation and detection of PCR products were performed with an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Allele frequencies, expected heterozygosities, exact tests of Hardy–Weinberg equilibrium, were all assessed with Arlequin v3.5 software. Statistical parameters to evaluate the forensic efficiency, such as discrimination power (DP) and random match probabilities (RMPs) for each locus and profile were calculated using PowerStats software (Promega CO).

Observed heterozygosity values were in the range 0.1053–0.5702. After Bonferroni correction Y03 (rs10629077) is the only locus in Hardy-Weinberg disequilibrium (P = 0.0002). The combined power of exclusion (PE) was 99.62%. The highest discrimination power locus was R01 (rs2308137) (DP = 0.6524) and the combined discrimination power (DP) for the 38 indel-plex was 0.99999999999997 for this population, allowing an acceptable level of discrimination in forensic cases.

Genetic population data of 38 autosomal indels for the Amerindian community Embera-Chami of Lapo-Antioquia

J.J. Builes[1,2], J.M. Ospino[3], A Manrique[1,2], D.P. Aguirre[1], L Mendoza[1,4], M.L.J Bravo[1], R Pereira[5], L Gusmão[5]

[1] *Laboratorio GENES Ltda, Medellín, Colombia.*

[2] *Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.*

[3] *Hospital La Cruz, Segovia, Antioquia*

[4] *Universidad Pontificia Bolivariana, Medellín, Colombia*

[5] *IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal*

Colombia has many Amerindian communities. The Embera-Chami, a community of 25,000 individuals who live in northwest Colombia, is the community with the greatest territorial dispersion but with the lowest population density on the territories they occupy. The aim of this work is genotyping 38 non-coding bi-allelic autosomal indels in a sample of this population.

Samples of 79 Amerindians Embera-Chami of Lapo-Antioquia-Colombia were taken. The DNA was isolated by salting-out. It was used the 38 indel-plex developed by Pereira et al 2009. Separation and detection of PCR products were performed with an ABI PRISM 3130 Genetic Analyzer (AB) and whit a FMBIO IIe (Hitachi). Allele frequencies, expected heterozygosities, exact tests of Hardy–Weinberg equilibrium, were all assessed with Arlequin v3.5 software. Statistical parameters to evaluate the forensic efficiency, such as discrimination power (DP) and random match probabilities (RMPs) for each locus and profile were calculated using PowerStats software (Promega).

Of the 38 indels used in this work, the marker rs2307579 was the only monomorphic. Observed heterozygosity values, for the remaining indels, were in the range 0.0127–0.6582. Hardy–Weinberg equilibrium tests demonstrated no significant deviation from expected values (P > 0.0013, after Bonferroni correction for multiple testing) for all of the 37 InDel markers in the Embera-Chami population. The combined power of exclusion (PE) was 99.40%. The highest discrimination power locus was B07 (rs3047269) (DP = 0.6444) and the combined discrimination power (DP) for the 38 indel-plex was 0.99999999998 for this population, allowing an acceptable level of discrimination in forensic cases.

Kinship analysis based on IBD segment from SNP microarray data

K. Miyashita[1], Y. Inaoka[1], E. Ochiai[1], T. Tamura[1], F. Satoh[1], M. Osawa[1]

[1] *Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan*

The conventional STR multiplex system has the limited power in kinship analysis such as the relation between full siblings. The whole genome SNP data potentially overcomes the difficulty. The recently developed ERSA algorithm provided the length and number of shared IBD segments, which gives a new clue to relatedness investigation. In the present study, we evaluated the power of the ERSA system in pairwise comparison of various relations. SNP typing was performed using Affymetrix Genome-wide Human SNP array 6.0 (Affymetrix) according to the he manufacturer's instruction. The genotypes were determined for each individual by the Birdseed version 2.3 genotype calling algorithm, embedded in the Affymetrix Genotyping Console 2.0. Fragments longer than 1 cM were collected through contiguous segment lengths of 64, 76, 96 and 128 SNP numbers (bits). Distribution of the IBD segment was compared among pairwise samples of parent-offspring (n=11), full siblings (n=15), second degree (n=9) and third degree (n=2). The bit number was the key factor that affected the separation of the relatives from un-relatives, and specificity of one

relation to other relations. Precise confirmation of the relatedness was achieved only to ~70%. It should be caused by that the reference SNP data was not constructed for the Japanese. To improve the analytic power, the reference data should be re-constructed using that from the same ethnic group of subjects.

Developmental Validation of Mitochondrial SNP Assays for Adept Matrilineal Inference of Biogeographic Ancestry at a Continental level

L.Chaitanya[1], Oven M van[1], N.Weiler[2], J.Harteveld[2], L.Wirken[3], Titia Sijen[2], Peter de Knijff[3] & Manfred Kayser[1]

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

[2]Department of Human Biological Traces, Netherlands Forensic Institute, The Hague, The Netherlands

[3]Forensic Laboratory for DNA Research, Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

The maternally inherited mitochondrial DNA (mtDNA) genome, being haploid, of small size (~16,570bp), and high in cellular copy number, has intrigued the forensic researchers and is beginning to be used for bio-geographic ancestry prediction. SNaPshot mtDNA multiplex genotyping assays with 58 single nucleotide polymorphisms (SNPs) were previously developed for resolution of bio-geographic ancestry of an individual at the continental level [1,2]. The current study validates the assays following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. This hierarchical system of assays defines the broad geographic region of matrilineal ancestry of an individual and determines the major haplogroups present in Africa, America, Western Eurasia, Eastern Eurasia, Australia and Oceania. These assays are highly sensitive and can produce full profiles at very low concentration of 1pg of DNA and are hence more sensitive than several commercially available STR kits. It is highly robust and efficient in providing information from degraded samples and from simulated casework samples of different substrates such as blood, semen, hair, saliva and trace DNA samples. Reproducible results were achieved from concordance testing across three independent laboratories depicting its ease and reliability. With this forensically validated mtDNA tool it is now possible to determine the matrilineal bio-geographic ancestry from DNA samples often encountered at forensic crime scene and provide lead for an investigation when an STR profile is not obtained from limited or degraded samples or in the absence of hits within a criminal DNA database.

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Forensic SNP detection using molecular beacons

J.D. Fredericks[1], M.E. D'Amato[1], M. Meyer[1]

[1]University of the Western Cape, Private Bag X17, Bellville, 7537, South Africa

There has been considerable debate regarding whether DNA analysis in forensic science has stagnated in terms of innovation and development. PCR, a relatively time-consuming protocol and STR (Short Tandem Repeats) analysis are still dominant processes in DNA analysis. SNPs (Single Nucleotide Polymorphisms) occur approximately every 1000 base pairs in the human genome and show unquestionable advantages over STRs, particularly in cases of highly degraded DNA (natural disasters, victims of war, mass graves, etc). Bi-allelic SNPs may have a lower power of discrimination when compared to multi-allelic STRs. However, their fragment size required for analysis is relatively small in comparison. The aim of this study is develop a molecular beacon multiplex that would be able to genotype several SNPs. A multiplex that will reduce the level of DNA integrity and time required for DNA analysis. Using 5-FAM or quantum dots (QDs) as a reporter dye and Iowa black® quenchers this study demonstrate the ability of molecular beacons to distinguish a single base mismatch in less than three minutes. Using the Rotor-Gene Q® platform the molecular beacons have successfully genotyped forensic SNPs from saliva, blood and bone DNA. Moreover, by increasing the sensitivity of the probe using QDs as a reporter dye, very low concentrations of DNA could be genotyped. This study demonstrates how molecular beacons can be used to rapidly identify forensic SNPs, while reducing the length of intact DNA traditionally required for analysis.

This project is funded by the Department of Science and Technology of South Afica (DST) andMintek, South Africa.

Analysis InDel markers using multiplex Pyrosequencing.

M. Allen[1], Bus, M[1]

[1]Uppsala University, Dept. of Immunology, Genetics and Pathology, Uppsala, Sweden

Introduction and aims: The aim of this study was to develop a genotyping method suitable for old/degraded samples, where the DNA template is highly fragmented and present in low copy numbers. To type this type of material, relatively short PCR amplicons and a robust sequencing method is required. Therefore, we have developed a novel assay for multiplex analysis of InDel markers using the pyrosequencing technology.

Materials and methods: A total of eight InDel markers present in the Investigator® DIPplex Kit were chosen and a multiplex pyrosequencing analysis assay was designed. An average PIC value (0.356) indicated that the selected markers were highly polymorphic. The pyrosequencing technology is based on sequencing-by-synthesis, and constitutes an alternative to the standard Sanger method, opening new possibilities for highly significant application in several sequence-based analyses. To distinguish signals (presence or absence of deletions) of separate InDel markers in the pyrosequencing multiplex, we developed a suitable directed dispensation order. Tests on high quality DNA showed that it was possible to perform pyrosequencing analysis of up to five markers in one multiplex.

Results and discussion: The combined power of discrimination based on the variability observed in the eight InDel markers reached 0.999362676 and the cumulative probability of exclusion was 0.775596. Furthermore, tests on degraded DNA and challenging samples show a highly sensitive and robust assay. This assay and methodology allow a sensitive, robust, cheap and fast sequencing method, ideal for analysis of smaller sample sets for forensic individual identification, analysis of museum specimens and ancient DNA typing.

Individual assignment of body fluids in mixed stains by synonymous SNP analysis

M. Omedei[1,2], S. Gino[1], S. Inturri[1], S. Pasino[1], C. Robino[1]

[1] Department of Public Health Sciences and Pediatrics, Laboratory of Criminalistic Sciences, University of Turin, Turin, Italy [2] University of Study of Rome "Tor Vergata", Department of Public Health, Rome, Italy

Messenger RNA (mRNA) profiling is a promising alternative to protein-based tests for the identification of biological fluids, and the availability

of RNA/DNA co-extraction methods makes it fully compatible with DNA analysis in forensic casework. However, when mixed stains containing different body fluids from multiple donors are found, it is impossible to determine, by conventional DNA testing, what cellular type each subject contributed to the mixture.

Genotyping of transcribed single nucleotide polymorphisms (SNPs) in mRNA derived cDNA molecules can possibly overcome this limitation. Consequently, we screened blood-specific target genes reported in the forensic literature for synonymous SNPs with no known clinical significance and minor allele frequency >25% in major human populations: marker rs857870 in the myeloid cell nuclear differentiation antigen (MNDA) gene was selected for preliminary study.

Two single base extension (SBE) assays for MNDA genotyping were designed, one targeting genomic DNA, another using cDNA specific primers spanning exon–exon junctions. Pairs of volunteer donors with informative MNDA genotype were identified and two-person stains created, mixing different ratios of blood from one donor with non-blood (semen/saliva) volumes from the other. After DNA/RNA co-isolation, DNA was subjected to routine STR typing, whereas RNA underwent DNase treatment and reverse transcription PCR, followed by SBE. In all the mixtures where DNA contribution from the blood donor was detectable by STR profiling, his MNDA genotype was obtained -either alone, or as major component- by SBE, showing the potential usefulness of the method for individual assignment of body fluids in mixed forensic stains.

Testing 86 SNPs performance for the simultaneous prediction of biogeographic ancestry (BGA) and pigmentation type

O. Bulbul[1], C Phillips[2], A. Freire Aradas[2], Y. Ruiz[2], T. Gross[3], T. Zorlu[1], G. Filoglu[1], H. Altuncul[1], Á. Carracedo[2], P.M. Schneider[3]

[1] Institute of Forensic Sciences, Istanbul University, Turkey

[2] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain

[3] Institute of Legal Medicine, University of Cologne, Germany

When an STR profile obtained from crime scene evidence does not match identified suspects or profiles from available databases, predictions of biogeographic ancestry (BGA) and phenotype inferences from DNA samples may be useful to the investigation. One of the goals of our study was to differentiate the ancestries of closely related Eurasian subpopulations using complementary forensic AIM-SNP sets. We developed a new 33-plex system to increase the resolution and informative value of two previously established forensic AIM-SNP multiplexes; 34-plex and Eurasiaplex. Another goal was the inclusion of pigmentation-informative SNPs to the 33plex system to enable predictions of externally visible characteristics. We re-designed the 33plex to include the additional pigmentation-informative SNP rs1129038. Minisequencing technology was used to analyze these panels combining a total of 86 ancestry and phenotype predictive SNPs. The reference SNP data from HGDP-CEPH and 1000 Genomes population panels were compared with 12 studied populations using STRUCTURE and PCA analyses. Results showed that individuals can be classified in the expected groups according to their BGAs with an improved differentiation of East Asian populations. We also applied the IrisPlex Excel calculator and SNIPPER analysis to predict the iris color of 100 Turkish individuals. Both tests provided over 90% accuracy with 6 SNPs for the prediction of blue vs. brown eye color. The likelihoods for green eye color predictions improved by including SNP rs1129038. However, further research is required to gather more information to improve the prediction of intermediate eye color.

Development of a 9 Mitochondrial SNPs Multiplex for Turkish population

H. Altuncul[1], O. Bulbul[1], I. Tavaci[1], A. Duvenci[1], Y. Demirbas[1], A. Katoglu[1], M. Olcen[1], D. Argac[1], G. Filoglu[1]

[1] Institute of Forensic Sciences, Istanbul University, Turkey

The use of mitochondrial DNA (mtDNA) typing in degraded and small amount of samples is rapidly increasing in forensic science. Genotyping of additional SNPs in the coding regions of mtDNA has been suggested for expanding the power of discrimination between individuals with identical HVR I and HVR II types. It is possible to analyze the samples by utilizing mitochondrial single nucleotide polymorphisms (mtSNPs) via minisequencing technique. The aims of this research are development of a forensic mtSNP multiplex and identification of polymorphic sites in Turkish population. 52 mtSNPs on coding region were selected from previous studies according to their variation in the Caucasians. 80 blood or buccal swabs samples were obtained from volunteers from Turkey. Optimization was done successfully by changing some assay parameters. mtSNPs were tested in 8 different multiplex with minisequencing technique using the Snapshot Multiplex kit (Applied Biosystems). We have determined polymorphism on 9 out of 52 mtSNPs (3010, 7028, 15340, 16519, 72, 12438, 11719, 15884, 12007) in Turkish samples. Based on consequences a new 9 mtSNPs multiplex was developed from previously selected 52 mtSNPs that can be used in various forensic applications for Turkish population. Therefore it is required to increase the number of study samples for reliable results and as well as add more polymorphic SNPs for increasing the discrimination power.

The SNPforID 34-plex – its ability to infer level of admixture in individuals

P.R.Prestes[1,2], R.J.Mitchell[1], C.Santos[3], R.A.H van Oorschot[2]

[1] Department of Genetics, La Trobe University, Melbourne, VIC, Australia

[2] Victoria Police Forensic Services Department, Melbourne, VIC, Australia

[3] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

Forensic scientists use genetic individualization markers to include or exclude persons of interest in investigations. However, when there are no suspects due to absence of database matches or eye-witness information, prediction of biogeographical ancestry can be a valuable investigative tool. The SNP*for*ID 34-plex uses 34 autosomal markers to predict ancestry from three geographic regions, Africa, Europe and Asia. However, it has a limited ability to identify levels of admixture within individuals.

We tested the 34-plex assay in 56 individuals from 15 families with varying levels of self-declared Asian-European admixed ancestry. STRUCTURE 2.3.4 was used for population structure analysis and cluster information provided inferences on levels of admixture. Chi-square tests were performed to evaluate the ability to predict level of admixture.

The average/±SD Asian and European contribution for individuals self-declared as first generation since admixture was 0.46/0.13 and 0.54/0.13, respectively. As expected, the average/±SD European contribution increased for individuals of 1/4, 1/8 and 1/16 Asian/European ancestries – 0.78/0.13, 0.89/0.05 and 0.91/0.03 respectively. There were no statistically significant differences between observed and expected average contribution from each ancestry. However,

individual outliers were observed which could have been misclassified if analysed separately.

These results suggest the 34-plex can be a reliable tool to predict levels of admixture, however caution is required when an individual sample is investigated. A larger number of markers, combined with increased sample sizes comprising varying levels of admixture and different genetic backgrounds, is required to enhance our ability to predict an individual's levels of biogeographical ancestry.

SNPs genotyping of forensic casework samples using 52 SNPforID markers

S. Alimat[1,2] S. Hadi[1], W. Goodwin[1]

[1]School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, United Kingdom.

[2]Forensic Division, Department of Chemistry Malaysia (KIMIA), Ministry of Science and Technology, Malaysia.

The analysis of degraded DNA is one of the biggest challenges in forensic casework. SNPs, which can be amplified using small amplicons, have been successfully applied to the profiling of forensic evidence that could not be analyzed using conventional STRs. We selected the 52 SNPforID markers, with amplicons that ranged in size from 59 bp to 115 bp, and applied them to the profiling of casework samples from Malaysia, where DNA degradation is a common problem due to the high temperatures and levels of humidity. To carry out the study we modified the 52 SNPforID markers into four 13-plex SNaPshot assays to enable easier interpretation of profiles on the ABI PRISM® 310 and 3500.

Fifty-one crime samples comprising bloodstains on cloths, swabs, and a mat and 2 swabs of trace DNA from 10 crime scenes in Malaysia were profiled after extraction of DNA using a phenol-chloroform method. The samples were also subjected to STR analysis using the Powerplex® 16 system (Promega), which resulted in only 17 full profiles. . Using SNPs,19 full profiles could be analyzed successfully from 34 samples that gave no or partial profiles with STR analysis.

Investigation of parent-of-origin SNPs in 5 imprinted genes for forensic purpose

S.H. Zhang[1], C.T. Li[1], S.M. Zhao[1]

[1]Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, P.R. China, Shanghai, P.R. China

Discriminating the parental origin of an allele may in some cases contribute not only to parentage test and personal identification in forensic casework, but also to other problems including the diagnosis of gender-related disorders. It is assumed that the monoallelic expression of the imprinted gene is caused by the existence of a differentially methylated region (DMR) and the gene is expressed on the hypomethylated allele, but exclusively suppressed on the hypermethylated allele. In this study, 6 Single Nucleotide Polymorphism (SNP) and their near regions on 5 imprinted gene were investigated. SNP of rs220028 and rs12916854 on SNRPN, rs737380 on SIM2, rs2279533 on ZNF215, rs760087 on COL9A3, rs2074399 on EVX1 were choosed based on databese of dbSNP (build 133) and geneimprint. The gDNA was extracted from peripheral blood of 30 healthy individuals and 10 trio families by QIAamp DNA mini kit (Qiagen). PCR and sequencing primers were designed by software of PyroMark Assay Design (Qiagen). Metylation of CpG dinucleotides and sequencing infomation of targeted regions were investigated by bisulphite treatment of gDNA, PCR and sequencing by PyroMark Q96(Qiagen). All the CpG dinucleotides were either methylated or not, which support the idea that the parental origin of an allele may be deternined by the methylation status of the allele. Besides, SNPs in near regions of targeted genes were with high discrimination power for forensic application. Research supported by the Ministry of Justice, P.R. China (#2011-2).

Combined analysis of two different ancestry informative typing kits using SNPs and Indels in Eurasian populations

T. Gross[1], D. Zaumsegel[1], M.A. Rothschild[1], P.M. Schneider[1]

[1]Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany

Current methods of DNA analysis usually require a comparative analysis of DNA profiles of crime scene samples with those of possible suspects or DNA database matches. Ancestry informative markers (AIMs) can provide valuable information in such special forensic cases, as they enable the inference of biogeographic ancestry. The knowledge of an unknown stain donor's biogeographic ancestry can be helpful in guiding criminal investigations.

The research focus for AIMs has been on multiplex assays of single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (Indels). SNPs and Indels share the advantageous properties for sensitive DNA analysis such as short amplicon sizes and high multiplexing capability.

To increase the informative value and resolution for Eurasian populations a combined analysis of a recently published Indel multiplex (21-plex, Zaumsegel et al., *FSI Genetics 7[2], 2013, 305*) and an in-house developed SNP multiplex for resolving Eurasian ancestry (31-plex) with an overall number of 52 markers was performed. Indel genotyping was done by a multiplex PCR with fluorescently-labelled primer pairs whereas SNP genotyping was based on SNaPshot technology. For the prediction of biogeographic ancestry an online Bayesian classification system (*Snipper*) was used.

A preliminary *STRUCTURE* analysis of Eurasian populations (Germans, French, Italians, Russians, Chinese, Vietnamese and Iraqis) resulted in an increased differentiation between the major continental groups when both multiplex assays were combined for analysis. The inclusion of additional sample sets from the HGDP CEPH human genome diversity panel will allow a better assessment of the improvement in predictive performance. Sensitivity and reproducibility as well as prediction error rates are addressed and discussed.

Population data for 30 insertion/deletions polymorphism in a Lithuanian population

V. Ivanova[1], J. Jankauskiene[2], D. Bunokiene[2], C. Børsting[3], N. Morling[3]

[1]Faculty of Natural Science, Vytautas Magnus University, Kaunas, Lithuania

[2]The State Forensic Medicine Service under the Ministry of Justice of the Republic of Lithuania, Vilnius, Lithuania

[3]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Human identification in forensic genetic case work is usually based on the analysis of short tandem repeats (STRs) or single nucleotide polymorphisms (SNPs) depending on the specific characteristics of the investigation. Analysis of biallelic insertion/deletion (indels) polymorphisms combines the advantages of both STR and SNP analyses: simple fragment analysis, short amplicon size ranges, high multiplexing capability and low mutation rates.

This study reports on the genotyping of 110 unrelated individuals from Lithuania with the Investigator® DIPplex kit (Qiagen). The kit amplifies 30 biallelic indels in one multiplex PCR. The indels are detected by standard fragment analysis using capillary electrophoresis. Full indel profiles were generated from 2 ng of DNA. The combined mean match probability was 3.3×10-13, the mean paternity exclusion probability was 99.7% and the typical paternity indices for trios and duos were 2360 and 166, respectively.

In conclusion, the Investigator® DIPplex kit can be useful in daily case work in the forensic laboratories.

SNP-STR Polymorphism: A Sensitive Compound Marker for Forensic Genetic Applications

W. Liang[1,2], P.M. Schneider[2], M. A. Rothschild[2], P. Bai[1], L. Zhang[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, PR China

[2]Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

Many routine STR and SNP multiplex kits can analyze a large number of markers but can not type STRs and SNPs in the same reaction. We have explored a method for typing STRs and SNPs in a single reaction named SNP-STR that links SNPs from a flanking region with the STR polymorphism. This allows to define subtypes of STR alleles based on the linked SNP allele observed in the flanking region.

Pairs of SNPs and STRs linked to each other and at a distance of less than 500bp were selected from the UCSC genome browser. For the STR D5S818 we designed two forward SNP allele-specific primers which are labeled by different fluorescent dyes, and with the reverse primer located at the other side of the STR sequence. The genotyping techniques are the same as for routine STR profiling. Samples from 95 unrelated European individuals, as well as an artificially generated unbalanced two-person DNA mixture were tested for the rs25768-D5S818 combination.

Both the discrimination power and the exclusion power of rs25768-D5S818 are higher than D5S818 alone. We also obtained a DNA profile with good peak heights from the minor DNA (25pg, 2.5%) of the mixture where the two sample donors have opposite homozygous alleles of rs25768. The advantages of the new SNP-STR markers are: first, the forensic efficiency of SNP-STRs is higher compared to standard autosomal STRs; second, allele-specific primers can be used to detect the minor DNA component with higher sensitivity than normal STRs; third, the application of the SNP-STRs can be improved because they are widespread in the genome; fourth, the difference in mutation rates of SNPs and STRs may have valuable information in evolutionary studies when SNP-STRs are applied.

Genetic Data for 29 INDELs and Amelogenin in 5 Chinese Populations with Qiagen Investigator DIPplex Kit

W. Liang[1,2], D. Zaumsegel[2], M.A. Rothschild[2], M. Lv[1], L. Zhang[1], J. Li[3], F. Liu[4], J. Xiang[4], P.M. Schneider[2].

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, PR China

[2]Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

[3]Guangxi Insititute of Criminal Science and Technology, Liuzhou Public Security Bureau, Liuzhou, PR. China

[4]Leshan Public Security Bureau, Leshan, PR. China

Insertion/deletion polymorphisms (INDELs) are diallelic markers with characteristics similar to SNPs such as low mutation rates. INDELs combine advantages of both STR and SNP markers. They have very small amplicon sizes and the genotyping techniques by fragment length analysis are the same as for routine STR profiling, which makes them suitable for personal identification and parentage testing. In our study, we genotyped 5 Chinese populations, including 73 Han population, 90 Zhuang population, 60 Dong population, 55 Miao population and 65 Tibetan population using the Qiagen Investigator DIPplex kit (QIAGEN, Germany) to perform a population study. The DIPplex marker set contains 30 INDELs and amelogenin, and is the first commercial kit available.

Blood stain samples were extracted using the EZ1 Advanced Instrument (QIAGEN, Germany) 500pg of DNA were amplified and products were analyzed using an AB 3130 capillary instrument (Applied Biosystems, USA). The results and data were analyzed using Genemapper ID and ARLEQUIN statistical software v.3.5.

All 30 loci fulfill Hardy-Weinberg equilibrium after Bonferroni correction. In Chinese Han population, the power of exclusion of individual markers ranges from 0.0123 to 0.2474, and the combined power of exclusion is 0.9892. The combined power of discrimination is 0.999999991. All resulting population genetic data are currently analyzed and compared regarding all relevant biostatistical parameters. We can conclude that the DIPplex kit is useful as an additional panel of genetic markers in Chinese populations.

Investigation of the genetic polymorphism of 30 InDel markers in DIPplex kit in the Hebei Han populations

X. Zhang[1], G. Fu[1], S. Li[1], L. Fu[1], B. Cong[1]

[1]Department of Forensic Medicine Hebei Medical University, Shijiazhuang, Hebei, People's Republic of China.

Introduction and aims Insertion–deletion (INDEL) markers are very frequent in the human genome and present several advantages for population and forensic studies, such as low mutation rates, small amplicons, easy genotyping. The aims of the present study were to investigate and evaluate the genetic polymorphism of **3**0 InDel markers in DIPplex kit in the Hebei Han populations.

Materials and methods In the present study, we genotyped 131 unrelated individuals living in Hebei in China using the Investigator DIPplex® kit (Qiagen). The values of observed heterozygosity (Ho), power of discrimination (PD), power of exclusion (PE) and polymorphism information content (PIC) were calculated. We also genotyped 34 trios to analyse DIPplex kit suitability for parentage testing.

Results and discussion Full indel profiles were generated from as little as 200 pg of DNA. The combined power of discrimination was 0.9999999988456 and the cumulative probability of exclusion (CPE) was 0.9812. All of the 30 InDel markers were in accordance with the Hardy-Weinberg equilibrium (P>0.0017, after Bonferroni correction for multiple testing). Combined paternity index reached about 2500. The data support that DIPplex kit is useful as an additional panel of markers in paternity cases when mutations in STR polymorphisms are present.

Validation of a multiplex system with 20 tri-allelic SNP loci for forensic identification purposes

Z.H.Li[1], J.Yan[1], D.Z.Tang[2], H.Zhu[1], Q.H.Bai[2], P.Y.Chen[1], Y.P.Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

[2]China National Accreditation Service for Conformity Assessment, Beijing100062, Beijing, China

Tri-allelic SNP has been regarded as a potential marker in forensic utility. Compared with the conventional binary SNP marker, it has showed obvious advantage in the analysis of mixed and degraded DNA samples. Previously, we constructed a multiplex system with 20 tri-allelic SNP loci by the pyrosequencing (PSQ) method and SNaPshot technique. In the present work, validation studies were carried out to determine the efficacy and reliability of the multiplex assay for forensic identification. Species specificity, sensitivity, artificially degraded samples and mixture study were executed in our research, according to the FBI / National Standards and Scientific Working Group on DNA Analysis Methods (SWGDAM) guideline. The results demonstrated that the multiplex system was sensitive to 1ng of input DNA. The complete SNP profiles could be consistently detected from the degraded DNA. No profiles were obtained in the forensically relevant animals. Additionally, using the multiplex SNP system, the presence of a mixture of two DNA samples in a ratio up to 3:7 could be recognized reliably. In conclusion, the 20-tri-allelic SNP multiplex system was suitable for paternity testing and human identification in forensic science. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK26B06) and by the National Natural Science Foundation of China (81072510).

Theme 8: New Technologies and methodologies in forensic genetics

Development of a New Rapid Cycling STR System to Address the Challenges of the European Forensics Community

D. Storts[1], R. McLaren[1], J. Patel[1], J. Bourdeau-Heller[1], and A. MacPhetridge[1]

[1]Promega Corporation, Madison, WI, USA

The need for forensic laboratories to obtain more information in significantly less time from their STR analyses has increased steadily in recent years. The PowerPlex® ESX and ESI Fast Systems not only meet the ENFSI recommendation for DNA profile sharing across Europe, but they also utilize rapid cycling technology, so that amplification using these five-color systems can be done in less than 50 minutes. These kits are offered in four formats to accommodate various laboratory requirements and preferences, including the option to detect SE33. Additionally, the kits offer superior inhibitor tolerance and enhanced sensitivity to obtain full profiles from low-level DNA inputs and are sufficiently robust to genotype degraded DNA samples through the incorporation of mini STR loci. Each kit can be used for both casework and databasing samples.

In this presentation, we will provide an overview of these systems as well as a detailed summary of preliminary developmental validation data on overall performance, including but not limited to, sensitivity, concordance, resistance to inhibitors, mixtures, and reduced cycling times. Comparisons were done to the standard PowerPlex® ESI and ESX Systems and the results indicate that performance of the Fast versions were as good or better than the standard PowerPlex® ESI and ESX Systems, demonstrating that the PowerPlex® ESI & ESX Fast Systems can be a valuable solution to the challenges of both casework and databasing laboratories.

Key Words: STR, rapid cycling, European Standard Set (ESS), PowerPlex, mini STR, inhibitors, degraded DNA.

Second Generation 'Rapid DNA' Instrument with a Fully-integrated Multiplexed, Single-use Microfluidic Cartridge

Delphine Le Roux[1,2], Brian E. Root[3], Jeffrey A. Hickey[3], Carmen R. Reedy[3], Joan Bienvenue[4], James P. Landers[3], Luc Chassagne[2], Philippe de Mazancourt[2]

[1] Morpho (Safran), Osny, France

[2] Versailles-Saint-Quentin-en-Yvelines University, Versailles, France

[3] ZyGEM Horizon-MicroLab Horizon, Charlottesville, Virginia, United States

[4] Lockheed Martin Corporation, Bethesda, Maryland, United States

DNA analysis is currently considered to be the gold standard for human identification (HID). Currently, the tendency is to reduce time-to-result, cost and analyst time consumed in processing reference samples. This would free the analyst for more complex casework and allow forensic labs to not add to the current backlog. It is for this reason that ZyGEM and Lockheed Martin have jointly developed an instrument capable of generating DNA results from buccal swabs in less than 90 minutes [1], exploiting microfluidics in a single, disposable cartridge that facilitates fully-automated analysis of four samples.

The study aimed to evaluate the second generation Rapid DNA instrument for generating STR profiles of acceptable forensic quality. The chemistry anchoring the technology was a combination of ZyGEM enzyme-based DNA preparation, infrared-mediated PCR amplification, electrophoretic separation in a unique polymer and detection of the STR fragments in a plastic cartridge the size of a 96-well plate. It was critical that acceptable results be obtained across all forensic metrics (precision, accuracy, reproducibility, concordance, contamination, etc.) when compared to currently-accepted conventional processes.

To accomplish this, one hundred swabs were tested by Morpho and ZyGEM in two different laboratories using two different instruments with Promega PP18D amplification chemistry. While work continues to improve the data quality and robustness of the platform, results indicate that full integration of the laboratory processes into a single, disposable HID microdevice allows for automated sample-to-answer capabilities, and begins to deliver on the time, cost and analyst time promised by microfluidics.

[1] Root, B et al. A Multichannel Microdevice for PCR Amplification and Electrophoretic Separation of DNA. Proceedings of 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS), 2011, Seattle, WA.

High quality DNA from Human Remains obtained by using the Maxwell® 16 Automated Methodology

D.A. Silva[1], M. Desidério[1], J. Aquino[1] , S. Bernardo[1], A. Caiafa[1], F. Manta[1], E.F. Carvalho[1]

[1]DNA Diagnostic Laboratory of Institute of Biology at State University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

DNA molecules from old unearthed bones and teeth are usually obtained in a severe level of degradation or contamination which makes them not suitable for PCR amplification most of the time.

During the last two decades, a growing number of techniques has been experienced to maximize the quality and to reduce the risk of contamination of the DNA extracted from human remains and automated methodologies have been developed and introduced in some forensic laboratories. This work aimed to standardize conditions for extraction of DNA from human bones, teeth and muscle by using the Maxwell® 16 LEV system (Promega Corporation) and the DNA IQTM Casework Sample Pro Kit for Maxwell® 16. Aiming to enlarge the amount of DNA having quality characteristics suitable for PCR amplification of SNP, INDELS and STR loci with different multiplexes systems a slight modification on the Maxwell® 16 original protocol has been shown to be correlated to a higher yield of DNA. The DNA solutions were amplified by using commercial and non-commercial multiplexes kits. Afterwards capillary electrophoresis (ABI 3500), the products of amplification were analyzed and genotyped using the software GeneMapperTM (Applied Biosystems). Considering INDELS and STR genotying results as well as mtDNA sequencing of bone, teeth and muscle human samples, it was found that the quality of the DNA produced by the Maxwell® 16 was higher than that of the DNA molecules obtained by the phenol/chloroform extraction procedure.

Financial support: Justice Court and Public Prosecuting DNA Program, Faperj, CNPq and CAPES

Characterization of mutations and sequence variations in complex STR loci by next generation sequencing

S. Dalsgaard[1], E. Rockenbauer[1], C. Gelardi[1,2], C. Børsting[1], S.L. Fordyce[1] and N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[2]Faculty of Mathematical, Physical and Natural Sciences, University of Palermo, Palermo, Italy

Next generation sequencing technologies (NGS) are well suited for STR sequencing and may be an alternative to large scale CE-based STR typing in forensic genetics. In the last couple of years, we have developed a robust 454 FLX pyrosequencing method for STR sequencing on the high-throughput GS Junior System (Roche Diagnostics). Complex STR loci consist of interrupted repeat sequences where variations in repeat numbers may be observed in more than one sub-repeat unit within the loci. Several of the core STR loci used in forensic genetics are complex STRs. We have recently shown that a large variation in sequence composition exists in the complex D21S11 locus. Furthermore, we found that it is possible to study the mutational mechanisms of D21S11 by NGS and characterize specific mutational events. The aims of this study was (1) to investigate the sequence variation in four complex STR loci, D2S1338, D3S1358, D12S391 and HumVWA, and (2) to study the mutational mechanisms in Danish paternity cases where genetic inconsistencies were observed between one of the parents and the child.

Novel strategies to speed up differential extraction of trace evidence as well as gynecological swabs after medical forensic examination

J. Edelmann[1], C. Kunz[1], F. Götz[2], H. Schnerr[3]

[1] Institut für Rechtsmedizin, Leipzig, Germany

[2] Qualitype GmbH, Dresden, Germany

[3] Biotype Diagnostic GmbH, Dresden, Germany

In sexual-assault cases the large quantity of female material may prevent detection of male DNA. Forensic DNA-laboratories try to separate male from female material to increase the chances of obtaining the perpetrator's autosomal profile. A solution to this problem is differential DNA extraction, but there is so far no established best practice.

A novel economic mini spin column format utilizes a unique self-sealing filter compartment that prevents flow-through of different kinds of liquid during incubation. This even allowed an immunological pretest performed using the identical sample material. Thus improved all over efficiency and ensured a subsequent differential lysis would be meaningful. Differential lysis was carried out under mild conditions to separate the female DNA and harsher lysis conditions that break the spermatozoa within the same filter column and without necessity of sample carriage. The DNA lysates were further purified and used to generate autosomal STR profiles of both the victim and the perpetrator.

Furthermore, a rapid 60 minutes DNA-extraction method was established and compared to the standard approach. Female and male DNA-extracts were ready to be used for direct PCR-amplification without any DNA purification. The novel strategies demonstrate an improvement of crime solution rates and are qualified to speed up analysis of the rape-kit backlogs.

Sample Lysis and DNA Extraction for direct PCR-Amplification in Single Tube Assemblies for Accurate Forensic Profiling

J. Edelmann[1], C. Kunz[1], F. Götz[2], H. Schnerr[3]

[1] Institut für Rechtsmedizin, Leipzig, Germany

[2] Qualitype GmbH, Dresden, Germany

[3] Biotype Diagnostic GmbH, Dresden, Germany

The importance to improve the quantity and quality of DNA isolated from forensic samples is without controversy. Extraction of genomic DNA forms the first step of DNA profiling and its quality is most critical for all subsequent steps to increase the potential to obtain maximum information from downstream Short Tandem Repeat (STR) analysis.

A novel kind of extraction system was characterized that allows simple and quantitative separation of substrate from lysate in a one-tube assembly. This approach eliminates the manual lysate and substrate transfer steps, saving time and minimizing cross contamination and sample transposition events significantly.

A specifically developed lysis buffer system allowed crude lysates to be used directly for PCR without time-consuming genomic DNA purification. Standard sample preparation was so realized in 20 minutes differential extraction of mixed specimens in less than an hour.

Due to high DNA yields, the chance of a successful DNA-profile by downstream analysis was significantly increased. Thereby, simple handling allows time savings and higher throughput in a manual process to allow reliable improvement of crime solution rates. Furthermore, the universal DNA extraction system demonstrated the potential as an improved methodology to overcome the often claimed difficulties in differential extraction, thus, has the potential to work off backlogs of rape kits.

Performance of the RapidHIT™200

H.S. Mogensen[1], R. Frank-Hansen[1], B. T. Simonsen[1], N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

RapidHIT™200 (IntegenX) is an all-in-one instrument built to produce STR profiles of reference samples (buccal swabs) in 90 minutes with minimal hands-on time. The instrument carries out extraction of DNA, PCR amplification and electrophoresis in the work flow. The aim of this study was to investigate the performance of the RapidHIT™200 with

regards to analysis of buccal swabs and to explore the possibilities of employing the instrument for analysis of more challenging sample formats than buccal swabs.

Nineteen mouth swabs were collected from eleven individuals. Fourteen swabs gave full profiles, three swabs gave partial profiles and two swabs gave no result. All alleles were assigned correctly.

Serial dilutions of lymphocytes containing cells corresponding to 6 – 6,000 ng DNA were spotted onto cotton swabs. Full profiles were obtained in the range of 900 – 1,200 ng DNA. With lower and higher amounts of DNA, drop-outs of alleles and loci were observed. There was no obvious correlation between the amounts of input DNA and the peak heights of the alleles in the profiles. However, when the peak height was low the risk of obtaining a partial profile was increased.

A piece of muscle from an identification case in 2007 was thawed and rubbed with cotton swabs for 10, 20 and 40 seconds. The rapidHIT™200 analyses gave partial STR profiles with correctly assigned alleles.

In general, alleles were assigned correctly and the muscle experiment was promising. There was no correlation between input DNA and peak heights and the sensitivity of the instrument is lower than conventional STR analysis.

Enhancing the Sexual Assault Workflow: Testing of Next Generation DNA Assessment and Y-STR Systems

J. Ballantyne[1], E.K. Hanson[1], R. Green[2], A. Holt[2], C. Bormann Chung[2], and J. Mulero[2]

[1]National Center for Forensic Science, University of Central Florida, Orlando, FL USA

[2]Human Identification, Life Technologies, Foster City, CA USA

Sexual assault samples are among the most difficult sample types encountered by forensic laboratories. Typically, a sexual assault sample has multiple challenges including small quantity of male DNA, relatively high quantity of female DNA, and presence of PCR inhibitors. These factors make it difficult to obtain an interpretable male profile. Therefore, there is a need for a more robust, highly sensitive, and faster method for the assessment (i.e. quality and quantity) of DNA extracts to determine optimal downstream processing methods, as well as an improved Y-STR amplification system for profiling these difficult samples.

We tested newly developed tools, including a DNA quantification kit that exhibits high sensitivity, higher inhibitor tolerance and includes additional useful tools for the determination of DNA quality to inform downstream processing methodology. We also tested a new Y-STR kit with enhanced capabilities. This 27-marker kit permits rapid amplification of single source and complex casework samples.

These two next generation systems can generate an improved workflow for obtaining interpretable profiles from sexual assault samples. We have successfully used the next generation DNA quantification and assessment kit and Y-STR kit to obtain informative Y-STR profiles from challenging sexual assault sample types, including low quantity of male DNA, extended interval post coital samples and samples containing high amounts of female DNA. The utility of the new workflow in processing of sexual assault samples will be presented.

Multiplex High Resolution Melt (HRM) Messenger RNA Profiling Assays for Body Fluid Identification

E. Hanson[1], J. Ballantyne[1,2]

[1]National Center for Forensic Science, University of Central Florida, Orlando, FL USA

[2]University of Central Florida, Department of Chemistry, Orlando, FL USA

Traditional body fluid identification methods use a variety of technologically diverse techniques that do not permit the identification of all body fluids. Definitive identification of the biological material present can be crucial to a fuller understanding of the circumstances pertaining to a crime. Thus definitive molecular based strategies for the conclusive identification of forensically relevant biological fluids need to be developed. Messenger RNA (mRNA) profiling is an example of such a molecular based approach.

Current mRNA body fluid identification assays typically involve either capillary electrophoresis (CE) or quantitative RT-PCR (qRT-PCR) platforms, each with its own limitations. Both platforms require the use of expensive fluorescently labeled primers or probes. CE-based assays require separate amplification and detection steps thus increasing the time required for analysis. For qRT-PCR assays, only 3 or 4 markers can be included in a single reaction since each requires a different fluorescent dye. To simplify mRNA profiling assays and to reduce the time and cost of analysis, we have developed multiplex high resolution melt (HRM) assays that provide an identification of all forensically relevant biological fluids and tissues.

The HRM assays require only the use of unlabeled PCR primers and a single intercalating fluorescent dye (Eva Green). Each body-fluid specific marker can easily be identified by the presence of a distinct melt peak. Here, we describe the development and initial performance evaluation of the developed HRM assays. The initial results demonstrate the potential use of HRM assays for the rapid screening of biological evidence.

Centrifugal microfluidic reaction platform for application in forensic genetics

J. Naue[1,2], M. Keller[3], S. Lutz-Bonengel[1], D. Mark.[3,4], K. Pietsch[5], N. Schlauderer[1], G. Segelbacher[6], O. Strohmeier[3,4], F. von Stetten[3,4], U. Schmidt[1]

[1] Institute of Legal Medicine, Freiburg University Medical Center, Freiburg, Germany

[2] Department of Biology, University of Freiburg, Freiburg, Germany

[3] Laboratory for MEMS Applications, IMTEK- Department of Microsystems Engineering - IMTEK, University of Freiburg, Freiburg, Germany

[4] HSG-IMIT, Institut für Mikro- und Informationstechnik, Freiburg, Germany

[5] State Institute for Chemical and Veterinary Analysis of Food Freiburg, Freiburg, Germany

[6] Department of Wildlife Ecology and Management, University Freiburg, Freiburg, Germany

Rapid forensic DNA analysis by assay automation is of increasing interest. Microfluidic technologies can precisely control minute liquid volumes in microstructured channels and reaction chambers, and thus seem highly suitable for automation of biochemical assays. Up to now, approximately 1600 rpm were required for centrifugal microfluidic operations. In this study, a real-time-PCR based screening assay for the detection of common European animal families was transferred to a centrifugal microfluidic polymer foil disc ("LabDisk") that can be processed on a common Rotor-Gene cycler with a rotary speed of 400 rpm.

In the assay, real-time PCR with EvaGreen is followed by melt curve

analysis. A universal duplex pre-amplification (PA) of mitochondrial *12S rRNA* and *cytochrome b* fragments is automatically aliquoted into 14 reaction cavities including 12 specific nested PCRs as well as controls. Aliquoting is accomplished by the centrifuge-thermopneumatic effect; an interplay of centrifugal forces and thermopneumatics. Enzymatic or column-based purification cannot be adapted to this microfluidic format. Thus, PA primer inactivation was achieved by different annealing temperatures, and blocking primers.

The assay was successfully processed on the LabDisk using the rotational frequency and thermocycling available on the Rotor-Gene instrument. Sensitivity is comparable to the assay run in tubes on the same cycler. The assay workflow allows for complete processing after addition of the sample without any further pipetting, purification, or transfers from one reaction tube to another. As a next step, the performance of cartridges with pre-stored assay reagents ("ready-to-use") will be tested.

This research project was funded by Deutsche Forschungsgemeinschaft.

Maximizing mtDNA Testing Potential with the Generation of High-Quality mtGenome Reference Data

R.S. Just[1,2], M.K. Scheible[1,2], S.A. Fast[1,2], K. Sturk-Andreaggi[1,2], J.L. Higginbotham[1,2], J.M. Bush[1,2], M.A. Peck[1,2], J.D. Ring[1,2], T.M. Diegoli[1,2], A.W. Röck[3], W. Parson[3], J.A. Irwin[1,2,4]

[1]Armed Forces DNA Identification Laboratory, Armed Forces Medical Examiner System, 115 Purple Heart Drive, Dover AFB, DE 19902

[2]American Registry of Pathology, 15245 Shady Grove Rd., Suite 335, Rockville, MD 20850

[3]Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria

[4]Present address :Federal Bureau of Investigation Laboratory, 2501 Investigation Parkway, Quantico, VA, 22135, USA

Mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate, high quality reference population data for estimating the rarity of questioned haplotypes and, in turn, the strength of the mtDNA evidence. Available databases (SWGDAM, EMPOP) currently include information from the mtDNA control region, and are thus more than adequate to support commonly employed assays targeting the hypervariable regions of the genome. However, novel methods that quickly and easily recover coding region data are becoming increasingly available. These assays promise to both facilitate the acquisition of mtGenome (mtG) data and maximize the general utility of mtDNA testing in forensics. Unfortunately, the reference data required for comparison and routine application in forensic casework are lacking. Until appropriate randomly-sampled and high-quality mtG reference population data are available, the utility of these novel assays will be limited.

To address this issue, we have undertaken an effort to: 1) increase the large-scale availability of high-quality entire mtDNA genome reference population data, and 2) improve the information technology infrastructure required to access/search mtGenome data and employ them in forensic casework. This presentation will describe our progress towards the development of 450 complete, high-quality mtGenomes spanning three U.S. population groups, and database structure and query modifications to the publicly-available EMPOP database (http://empop.org/). With the large-scale availability of high quality entire mtGenome data, the value of mtDNA testing can be greatly improved and its full potential in forensic casework realized. This work was supported by the US National Institute of Justice.

Reduced Volume Direct Amplification of Blood Samples on FTA cards Using the AmpFlSTR® Identifiler® Direct PCR Amplification Kit

J.Y.Y. Tan[1], S.J. Lin[1], S.Z. Tan[1], B. Heng[1], C.K.C. Syn[1,2]

[1]DNA Profiling Laboratory, Biology Division, Applied Sciences Group, Health Sciences Authority, Singapore.

[2]Department of Biological Sciences, National University of Singapore, Singapore.

The use of the FTAÔ card is often the preferred method for reference DNA sample collection and storage. Previous processing of blood samples on FTAÔ cards required the washing of the FTAÔ punch to remove inhibitors of the PCR reaction prior to amplification. Though the purification procedure was automated, the entire process was time consuming. The Applied Biosystems AmpFlSTR® Identifiler® Direct PCR Amplification Kit is designed to allow direct amplification of the FTAÔ punch without the need for prior sample cleanup. The present study validated the use of the Identifiler® Direct Kit for direct amplification of FTATM blood samples in combination with the 3500xL genetic analyzer. In addition, a reduced amplification volume protocol was validated, allowing for a significant reduction in reagent use.

A total of 243 casework blood samples on FTAÔ cards were used to optimize the PCR cycle number (i.e. 25, 26 or 27 cycles) and establish stutter percentage thresholds. Extraction blanks and amplification negatives were used to establish the analytical threshold.

The DNA profiles obtained from 0.75 mm punches with an amplification volume of 10 µL at 26 amplification cycles (instead of the manufacturer-recommended 25 µL reaction volume) showed full concordance to previously obtained DNA profiles (with the Identifiler® PCR Amplification Kit). Stutter values, heterozygote peak height balance, and allele peak heights were in the optimal range. The results thereby supported the use of reduced amplification volumes in conjunction with the Identifiler® Direct kit for the direct amplification of FTATM reference blood samples.

Novel technique of dental tissue collection for ancient DNA extraction from teeth.

S. Gonzalez[1], V. Avidad[2], J. Castro[1], M. Salazar, N. Seijas[3], P. Zambrano[4], R. Gasperi[5]

[1] Genetic Forensic Laboratory, Ministerio Público, Venezuela

[2]Forensic Odontology, Ministerio Público, Venezuela

[3]Forensic Science Department, Ministerio Público, Venezuela

[4]Criminalistics Unit Against Human Rights Infringement Ministerio Público, Venezuela

[5]Direction of Technical Scientific and Investigative Advisory, Ministerio Público, Venezuela

DNA extraction protocols from tooth samples require the grinding of the sample. Aim: Evaluate a technique of DNA extraction on teeth, with the preserving of the external structure, for further analysis. Materials and methods: An intact (left lower second) molar, from a skeleton found after two years in an open area, was prepared and sterilized by several washes. The tooth was cut in a transverse section at the middle third of the root with a milling tool (Dremel) with a 0.5mm diamond blade, to expose the root canal. The materials used to perform the extraction of dental tissue (compound of cement and dentin) was dental instruments as Dappen reservoirs, impression materials (silicone), endodontic files from 15mm to 40mm, etc. The was tooth fixed on the silicones, where manually scraped on the canal, to obtain 100mg to 150mg of grinded, and then, the two fractions of the tooth were joined with light-cured glass ionomer cement, in its original shape. DNA extraction was made with PrepFiler with BTA,

quantification by Quantifiler Duo on 7500 Real-Time PCR system, autosomic profile with Identifiler Plus and allelic assignment on 3130xl Genetic Analyzer with GeneMapper software v3.2, all from Applied Biosystems. Results and conclusions: it was possible to determine the complete genetic profile of the sample, avoiding the possible interference of minerals inhibitors present in the crown of the teeth. The advantage of this technique is that allows the preservation of the external morphology of the tooth for future identification analysis and its reinsertion into the corresponding mandibular alveolus.

Validation of the DNAscan™ Rapid DNA Analysis™ System: Presentation of data

J. French[1], M. May[2], N. Moran[3], R. Selden[2]

[1] GE Healthcare, Piscataway, New Jersey, United States of America

[2] NetBio, Waltham, Massachusetts, United States of America

[3] GE Healthcare, Whitchurch, Cardiff, United Kingdom

Introduction and aims: The DNAscan Rapid DNA Analysis System, when fully integrated into the law enforcement environment, will increase the speed of DNA processing, minimize the crime lab DNA backlogs, and help lower the cost of law enforcement by providing a biometric link between a criminal activity and an individual being held in custody. The aim of this presentation is to proffer for the first time the results of system's performance verification testing and developmental validation.

Materials and methods: The performance verification testing and developmental validation studies were designed and conducted to evaluate the "swab in, profile out" system as a whole, including all reagents and the expert system software. Challenging samples such as those demonstrating off-ladder alleles and triallelic patterns were incorporated into the set of test samples. The developmental validation studies were performed in partnership with several laboratories across the world. The FBI's Quality Assurance Guidelines for validation will be followed and the primary studies include, but are not limited to, sensitivity, reproducibility, contamination, and precision.

Results and discussion: The results of the performance verification testing and developmental validation will be presented. Additionally, results of ongoing reagent stability studies will be presented to demonstrate long-term, room temperature stability of the BioChipSet™ Cassette which houses all reagents needed for the DNA processing. The presentation of the results will provide the forensic community the necessary information to make a data-driven decision about robustness and reliability of the DNAscan system for implementation in the crime laboratory and law enforcement environment.

Early validation studies on the RapidHIT™ 200 Human DNA Identification System

K. Elliott[1], H. Franklin[1], L. Hennessy[1], S. Gangano[1]

[1]IntegenX, California, USA

Significant advances in DNA analysis have enabled the successful closure of many criminal cases that would have previously been beyond resolution. As a consequence, DNA testing is now recognized as a critical component of modern law enforcement. However, whereas technologies for other evidence types such as fingerprints and drugs have advanced in recent years enabling use outside of the laboratory, providing intelligence right at the start of investigations, DNA has remained within the confines of the laboratory. Enabling Rapid DNA testing outside of the laboratory represents the next major advance in how DNA will contribute to law enforcement, homeland security, and defense. The IntegenX RapidHIT™ 200 is designed to enable safe and easy deployment outside of the laboratory, and operation by non-scientists. Producing full DNA profiles in 90 minutes, the RapidHIT system enables DNA-based intelligence and valuable leads to be obtained quickly at the start of investigations.

Validation of Rapid DNA systems presents several significant challenges. The measurement uncertainty approach used by many laboratories can be difficult to apply to a fully enclosed 'black box' system, and use outside of the laboratory may require adherence to additional standards (e.g., ISO17020) not familiar to the forensic geneticist.

We will present initial results of our developmental validation studies performed on the RapidHIT system, including reproducibility, precision, accuracy, sensitivity, and genotype concordance. Laboratories adopting the system need to consider factors such as the scope of the intended application, the end user, and the environment when conducting their own internal validation studies.

Early validation studies on the RapidHIT™ 200

L. Ives[1]

[1]Key Forensic Services, Norwich, UK

The forensic community is continually striving to improve the analysis of DNA, particularly with the development of more rapid and efficient processing techniques. One such solution is the IntegenX RapidHIT™ 200.

The RapidHIT integrates sample handling steps through DNA extraction, normalisation, amplification utilising a commercially available STR kit, separation and detection. The process is carried out in less than 2 hours offering a significant reduction in processing time compared to existing automated lines.

Here we present preliminary studies conducted by Key Forensic Services (KFS) on an early release version of the RapidHIT 200, including a study for use of the RapidHIT for rapid intelligence. This study comprised 114 buccal swabs from known donors and 16 blanks. The results were assessed for concordance with donor profile, profile quality, and observations of environmental or sample to sample contamination.

Full DNA profiles, containing all expected donor alleles, were obtained from 46% of samples and results suitable for intelligence led searching of the UK database were obtained from 81%. The blank swabs demonstrated no incidences of contamination.

In the initial validation, marked variability in peak height was observed, and there were several incidences of 'allelic drop-out'. These observations appear to be largely due to inefficiencies during DNA extraction and purification, and sub-optimal performance of the STR chemistry.

Protocols for improving robustness of the instrument, including more efficient lysis and the introduction of next generation STR chemistries, were implemented post validation and the impact of these in terms of profile quality will also be presented.

Development of the Investigator STR GO! Kits for direct amplification of reference samples

M. Scherer[1], B. Alsdorf[1], L. Bochmann[1], A. Prochnow[1], H. Engel[1]

[1]Qiagen GmbH, Hilden, Germany

Reference samples are typically of high quality and quantity and the outcome of a STR analysis is more predictable compared to an unknown casework sample. The analysis process of reference samples therefore can be streamlined by doing STR PCR directly from a FTA punch or a crude lysate of a buccal swab, eliminating

sample extraction and quantification. Based on the fast and highly robust Investigator STR Plus kits we have developed assays for direct amplification, the Investigator STR GO! Kits. Three assay formats have been developed, covering the CODIS set of markers, as well as the new European Standard Set (ESS) with our without SE33. Blood or buccal cells on FTA and other paper and swabs can be used as sample. Direct PCR amplification only takes about 45 – 54 min depending on the type of sample. For buccal swabs a convenient 5 min room temperature lysis protocol to obtain a crude lysate to be used as PCR template is provided. The assays give rise to balanced profiles and allow high first pass rates for all common reference samples. To overcome unbalanced amplifications due to rare mutations in the primer binding sites of vWA, D16S539 and SE33, new SNP primers have been introduced.

Development and use of a single-tube four dye, 21plex direct PCR autosomal STR loci amplification assay for human identification and relationship testing

C.W. Francischini[1], D.R. Sumita[1], M.R. Whittle[1]

[1]Genomic Engenharia Molecular, São Paulo, SP, Brazil

Short tandem repeat (STR) loci are currently the markers of choice for routine casework involving human identification and human relationship testing. Currently there are various different kits commercially available for the multiplex PCR amplification of these loci. Earlier kits enabled amplification of 13 autosomal STR loci but more recent versions permit the co-amplification of 18 loci, and latterly, of 24 loci. This increase in STR number reflects a demand for increased power of discrimination, especially in certain types of relationship testing where close relatives may need to be distinguished. In this work we describe the development and use of single tube multiplex PCR kit in which 21 commonly used (and including CODIS) autosomal STR loci, together with the amelogenin locus, are amplified. For relationship testing the amplification occurs directly from a single 1 mm filter paper disk containing dried blood or saliva, and the loci are amplified using primers with only four different fluorophores, and for forensic analyses extracted human DNA in aqueous solution is used. The alleles are separated and visualized using standard capillary DNA sequencers. An allelic ladder comprising the most prevalent alleles of the 21 loci in the Brazilian population is also used to control for electrophoretic mobility, together with the GeneMapper software for the rapid generation of genotype tables. Besides amplifying more autosomal STR loci than most kits presently available on the market, we have found this kit to satisfy our requirements for all parentage testing and in forensic casework

Funding support: FINEP

Doing more with less: Novel Techniques for DNA Analysis in Bombings and Terrorism

M Nilsson[1], M Allen[2], M Havsjö[3], L Edwinsson[3], and J Grånemo[2], M. Bjerke[2]

[1] Stockholm County Police, Forensic Unit, Stockholm, Sweden

[2]Uppsala University, Dept. of Immunology, Genetics and Pathology, Uppsala, Sweden

[3]AlphaHelix Molecular Diagnostics AB, Uppsala, Sweden

Introduction and aims: The aim of the project is to improve DNA analysis of cellular materials on bombs after explosions to combat terrorism. Novel procedures for sample preparation, DNA repair and next generation sequencing (NGS) have the potential to significantly impact forensic individual identification by allowing reliable DNA analysis of samples with low amount of degraded DNA after detonations.

Materials and methods: Explosive devices have been produced and handled in a structured manner to simulate forensic samples. All available residues from the devices following explosion have been collected for further analysis of biological traces. To improve analysis of low template amounts as well as degraded DNA optimisation of the extraction, PCR enzymes, handling of inhibitors, Rolling Circle Amplification and next generation sequencing using Illuminas MiSeq have been performed.

Results and discussion: Five different DNA extraction kits have been evaluated for their efficiency to recover DNA. Variation in the quantity of extracted DNA were seen when the different methods were compared using real-time quantification. Moreover, different enzymes have been evaluated for most successful amplification, which indicate that the KAPA2G Robust enzyme result in higher DNA amount compared to our most commonly used enzyme, Taq Gold Polymerase. To study the inhibitory effects, explosive standards have been added to samples to simulate traces typically found in cases with explosions. Samples with low DNA levels have also been successfully amplified using. A new, fast PCR-instrument that takes approximately 20 minutes for a complete run has further improved our analysis.

Funding support for research: VINNOVA.

Examining the Utility of Next Generation Sequencing as a Routine Forensic Analysis Method

N. Oldroyd[1], K. Stephens[2], J. Varlaro[2], and C.L. Holt[2]

[1]Illumina UK Ltd., Chesterford Research Park, Little Chesterford, Essex, UK

[2]Illumin Inc., 5200 Illumina Way, San Diego, California, USA

The utility of DNA technology for criminal investigations has resulted in DNA analysis becoming the cornerstone of contemporary forensic science. However, advances in genomics technologies have outpaced methods that were first introduced into forensic testing, the fundamentals of which have changed little in the last decade. The advent of national DNA databases and the utility of DNA testing for the identification of missing persons, kinship testing, ancestry investigations and other human identification applications have resulted in increased numbers of samples being profiled. When combined with the requirement for more comprehensive results from lower template amounts, this puts strain on the fixed capabilities of traditional capillary electrophoresis (CE)-based methods.

Forensic scientists worldwide are now investigating the value of Next Generation Sequencing (NGS) for forensic applications. Illumina's sequencing by synthesis (SBS) technology offers a massively parallel approach for simple and accurate sequencing of large numbers of PCR amplicons. In this paper, we describe how development of new instrument and chemistry options result in NGS methods now becoming a viable alternative to current CE-based methods for mainstream forensic analysis. We demonstrate how, by delivering data that span the genome, NGS systems can answer a wider range of questions in a single, targeted assay offering a variety of key improvements including relieving analysis limitations with respect to challenging samples such as complex mixtures, flattening the analyst's decision tree and reducing workflow complexity compared to current methods. Specific validation and implementation requirements for NGS methods in forensic laboratories will also be discussed.

Successful direct amplification of nuclear markers from a single hair follicle

R. Ottens[1], D. Taylor[2], D. Abarno[2], A. Linacre[1]

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

[2] Forensic Science SA, Adelaide, South Australia, Australia

We report on successful amplification of DNA profiles from single hairs. Direct amplification was used on the root tip of both anagen and telogen hairs using a standard commercial forensic PCR kit to amplify 15 STR loci. All 30 anagen hairs tested from five different people gave full DNA profiles after 29 cycles with no allelic drop-in or increase in stochastic effects compared to using the standard extraction methods. Six of the 30 telogen hairs tested resulted in a full DNA profile, and a further four telogen hair samples tested produced a DNA profile of five or more complete loci that could be up-loaded to the National Criminal Investigation DNA Database (NCIDD, Australia). A full DNA profile was also obtained from the shaft of single anagen hairs. Current practice for many laboratories is that single telogen hairs are not subjected to DNA testing and anagen hairs are seldom tested as there is little chance of generating a meaningful DNA profile; hence this 100 % success rate in generating a DNA profile from anagen hairs is a significant advancement. The process described was trialled on current DNA profiling kits, using the manufacturer's recommended methods and no increase in cycle number, such that the methodology can be incorporated readily into a practicing forensic laboratory. For the first time in the field of human identification, single hairs can be analysed with confidence that a meaningful DNA profile will be generated and the data accepted by the criminal justice system.

Funding for Renée Ottens and Adrian Linacre was provided by the Department of Justice South Australia.

Do the amount of Proteinase K and the incubation time of samples make a difference in DNA extraction from bones? A comparative study of protocols

S. T. G. Ferreira[1]

[1]Instituto de Pesquisa de DNA Forense – Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil

In many protocols of DNA extraction, the components of extraction buffers, incubation time and Proteinase K are important variables for DNA yields and DNA typing results. The aim of this study was to compare if the amount of Proteinase K and the incubation time of samples make a difference in two protocols of DNA extraction from bones. We made a comparative study with these variables using modified organic phenol-chloroform protocol and the standard extraction protocol described in Edson et al, 2004. We selected 20 bone samples from femurs (13 from buried human remains and 7 from not buried skeletal remains). Each bone sample was prepared and powdered. 2 grams of each powdered bone were incubated in buffer extraction of each protocol with 24 and 48 hours of incubation time. In the 48-hour protocols the amount of Proteinase K was doubled in each sample 24 hours after the incubation. DNA was extracted, purified and concentrated. Real-time quantification and STR amplification were performed for each DNA extract. In this study, the efficiency of the amount of Proteinase K and the incubation time of samples were compared according to the amount of DNA yield and to the quality of DNA typing results in each protocol. Modified organic phenol-chloroform protocol with 24 hours of incubation time was the one which yielded higher amounts of DNA and better DNA typing results.

DNA Investigative Lead Development from Blood and Saliva Samples in Less than Two Hours Using a Fully Automated Rapid DNA System

S. Gangano[1]

[1]IntegenX, Pleasanton, California, USA

Blood and saliva samples are valuable for the development of investigative leads and are commonly recovered from many crime scenes. These samples, if processed quickly can dramatically accelerate the investigation and conclusion of crimes. However, traditional methods for DNA analysis are time and resource consuming. As a result, samples collected from many crime scenes do not return results until long after the early, and most crucial stages of an investigation have passed.

The RapidHITTM produces DNA profiles in 90 minutes and is designed for ease of use and ease of deployment, potentially at the crime scene itself. Until recently the RapidHIT was designed for reference samples such as buccal swabs. Here we present data from a protocol designed for blood stains and saliva samples (e.g. cigarettes and drinks cans).

Results obtained from a variety of bloodstains, and from a range of saliva samples typically recovered from crime scenes are presented. 88% of bloodstains produced results of sufficient quality for searching against a database (7 or more full loci, ensuring a sufficiently discriminating profile to avoid high numbers of adventitious matches). For saliva samples around 50ng of DNA was required for full profiles, with the majority of drinks cans and cigarette butts giving either full profiles or good partial profiles.

Using the RapidHIT, DNA results can now be obtained from a variety of crime samples, in a time frame that allows Law Enforcement to narrow the field of suspects and establish probable cause while the investigation remains fresh.

Theme 9: Forensic DNA phenotypic testing

The generics of eye clolours in an italian population measured with an objective method for eye colour quantification

Pietroni, C[1], Andersen J.D[1], Johansen P[1], Harder, S[2], Paulsen, R[2], Børsting C[1], Morling N[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

[2]DTU Informatics – Informatics and Mathematical Modeling, Technical University of Denmark

The brown and blue eye colour is primarily explained by the single nucleotide polymorphism (SNP), rs12913832. However, the genetics behind eye colours that appear to be neither blue nor brown is not well understood.

In this study, 240 unrelated Italians were typed for 32 SNPs located in pigmentary genes, including *HERC2, OCA2, TYR, SLC45A2, SLC24A5* and *IRF4*. High resolution digital images of the participants' eyes were taken and the iris regions were extracted with the use of the custom designed software Digital Iris Analysis Tool (DIAT). The software counted the numbers of blue and brown pixels in the iris images and calculated a Pixel Index of the Eye (PIE-score) that described the eye colours quantitatively. The PIE-score ranged from -1 to 1 (brown to blue). Associations between the PIE-score and the SNP types will be presented. Also, the performance of the DIAT software will be presented. With the use of the DIAT software and the PIE-score, it is possible to compare the iris colour of large numbers of iris images obtained in different studies and to perform large meta-studies that may reveal loci with small effects on the eye colour.

Development of predictive tests for skin and hair colour

O. Maroñas*[1], J. Söchtig*[1], C. Phillips[1], Y. Ruiz[1], R. Cruz[2], A. Gomez-Tato[3], M. Casares de Cal[3], J. Alvarez-Dios[3], Á. Carracedo[1,2], M.V. Lareu[1]

[1] Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Galicia, Spain.

[2] Genomic Medicine Group, University of Santiago de Compostela, Spain.

[3] Faculty of Mathematics, University of Santiago de Compostela, Spain.

** Contributed equally to the study*

To develop forensic pigmentation predictive tests we have analyzed 63 SNPs in three SNaPshot assays collating the SNPs with strongest associations to common Skin-Hair-Eye Pigmentation variation published in the recent literature, we termed the 'SHEP' discovery panel (Ruiz et al., 2013). Using the SHEP panel, data from SNPs in nine skin pigmentation-associated genes was used to predict skin colour within Europeans and between Europeans and Africans. More than 300 individuals were analyzed comprising mainly Spanish and Scandinavian/German Europeans plus Africans from Senegal. Individuals were measured by reflectometry and skin tones were recorded photographically. To facilitate adoption of SNP based skin colour predictive tests in forensic applications we have also adapted the *Snipper* naïve Bayesian classifier, originally developed for ancestry analysis, in order to provide a straightforward system to assign skin colour likelihoods from a profile of the most strongly associated SNPs.

For the analysis of hair colour variation, more than 400 European samples were obtained from Germany, Spain, Scandinavia, Austria and Italy, representing the wide hair colour variation in Europe. Phenotyping was accomplished using a graded colour category system of reference shades plus photography. The predictive system also applied the adapted *Snipper* classifier to provide hair colour assignment probabilities for the most strongly associated SNPs. Results show the best estimated success ratios apply to fair (blond) and dark haired (black) individuals, while intermediate hair colours give reduced predictive success.

Y. Ruiz, C. Phillips, A. Gómez-Tato, J. Álvarez-Dios, M. Casares de Cal, R. Cruz, O. Maroñas, J. Söchtig, et al., Further development of forensic eye color predictive tests. Forensic Sci Int Genet. (2013) 7: 28-40.

Exploring iris colour prediction and ancestry inference in admixed populations of South America

A. Freire-Aradas[1], Y. Ruiz[1], C. Phillips[1], O. Maroñas[1], J. Söchtig[1], V.N. Silbiger[2], A.D. Luchessi[2], A. Luchessi[3], Á. Carracedo[1], M.V. Lareu[1]

[1] Forensic Genetics Unit, University of Santiago de Compostela, Spain.

[2] Facultade de Farmácia, Universidade Federal do Río Grande do Norte, Natal, Brazil.

[3] Facultade de Ciências Aplicadas, Universidade Estadual de Campinas, Limeira, Brazil

Forensic Genetics is entering a new phase with the development of DNA-based predictive tests for physical characteristics, such as pigmentation traits, together with inference of genetic ancestry using single nucleotide polymorphisms (SNPs). Hair and eye colour variation is largely confined to European populations and although one SNP in particular, rs12913832 in HERC2, is responsible for the greatest proportion of eye colour predictability, several other SNPs in five other genes are also informative for differentiating blue and brown iris phenotypes and have been brought together in the Irisplex test [Walsh et al, 2011]. Since multiple SNPs in combination, not a single marker, contribute to eye colour predictability in Europeans, it is likely that the predictive performance of Irisplex or extended SNP combinations [Ruiz et al., 2013] will be different in admixed populations that have European co-ancestry compared to unadmixed Europeans.

In this study we examined two admixed South American population samples from Brazil and Venezuela to assess the predictive performance of an extended SNP set of 23 markers [Ruiz et al, 2013]. Many of these additional SNPs are weakly predictive but we previously indicated they can contribute to intermediate eye colour predictions. We assessed levels of European, Native American and African co-ancestry in the sampled populations by genotyping 61 autosomal ancestry-informative marker SNPs, a large proportion of which were developed to improve differentiation of American from European and African ancestries. We observed correlations between the proportion of European ancestry and 'light' eye colour variation (i.e. non-brown).

Acknowledgements: This project was supported by funding from Xunta de Galicia INCITE 09 208163PR (MVL) and a mobility grant from the Asociación Universitaria Iberoamericana de Postgrado (AFA).

Dropout probabilities of irisplex SNP alleles

Andersen J.D[1], Tvedebrink, T[2], Mogensen, H[1], Børsting C[1], Morling N[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

[2]Department of Mathematical Sciences, Aalborg University, Denmark

In certain crime cases, information about a perpetrator's phenotype, including eye colour, may be a valuable tool if no suspect or individual in the DNA database match the STR profile found at the crime scene. Often, the available DNA material is sparse and allelic dropouts of true alleles are possible. As part of the validation of the IrisPlex assay in our ISO17025 accredited, forensic genetic laboratory, we estimated the dropout probabilities per SNP locus using different numbers of PCR and Single Base Extension (SBE) cycles.

The six IrisPlex SNPs were amplified in a single PCR multiplex and detected by one SBE multiplex, capillary electrophoresis (CE) and multi-colour fluorescence detection on an LT/AB 3130xl DNA sequencer. A total of 19 different samples were each typed using five different amounts of input DNA ranging from 500pg to 31pg. Controlled experiments were carried out using 29 and 30 PCR cycles and 25, 50 and 100 SBE cycles.

In order to model the drop-out probabilities for a given sample, the SNP allele imbalances were modeled and incorporated in the estimate of the underlying signal intensity. We used a mixed effects model to capture the fixed SNP and locus effects, and we used a random effects model to adjust for DNA profile effects.

The drop-out probabilities depended on the overall signal intensity. Locus and allele specific parameters were estimated.

Eye colour SNPs variants in Thai population.

K. Vongpaisarnsin[1], K. Vongpaisarnsin[2]

[1]Forensic DNA and serology, Department of Forensic Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[2]Metta International Eye Center, Mettapracharak(Wat Rai Khing) Hospital, Nakhon Pathom, Thailand

Human eye colour is recognized as one of the visible characterized features that would be valuable for forensic identification by predicting these phenotypes with particular genotypes. Recent gene association study had figured the remarkable variants mainly in *OCA2* and *HERC2* genes.

The objective of this study is to present the eye colour SNPs variants and major haplotype found in Thai population.

Eight human eye colour single nucleotide polymorphisms (SNPs) in five pigmentation genes, *OCA2* (rs7495174, rs4778241 and rs4778138), *HERC2* (rs12913832 and rs1667394), *SLC24A4* (rs12896399), *SLC45A2* (rs16891982) and *TYR* (rs1393350) were investigated in 374 Thai individuals.

The allele frequency comparison with other populations in HapMap had identified the major allele of East Asia and Thai. The homozygote genotypes were predominantly observed in rs16891982 (GG/0.99198), rs1393350 (GG/0.99733) and rs12913832 (AA/0.98663). Furthermore, the analysis of haplotype block of HERC2-OCA2 comprised five SNPs (rs12913832, rs1667394, rs7495174, rs4778241 and rs4778138,) presented the most haplotype was AG-GAG (0.5199), which was rarely observed in European population.

Association of SNPs from the SLC45A2 gene with human pigmentation traits in Brazil

N. C. A. Fracasso[1], E. S. Andrade[1], C. C. F. Andrade[1], L. R. Zanão[2], M. S. Silva[2], L. A. Marano[1], C. E. V. Wiezel[1], E. A. Donadi[3], A. L. Simões[1], C. T. Mendes-Junior[2].

[1] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil;

[2] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil;

[3] Divisão de Imunologia Clínica, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo - USP, Ribeirão Preto, SP, Brazil;

The SLC45A2 gene encodes the Membrane-Associated Transporter Protein (MATP), which mediates melanin synthesis by tyrosinase trafficking and/or proton transportation to melanosomes. Two coding SNPs (E272K and L374F) in this gene have been associated with variation in human pigmentation. Since the determination of eye, hair and skin pigmentation of unknown samples found in crime scenes would be of great value for forensic caseworks, the present study aimed to further investigate the polymorphisms of the SLC45A2 gene and their alleles or genotypes associations with such pigmentation traits in a highly admixed population sample.

To achieve this goal, 12 SLC45A2 SNPs (Yuasa et al., 2006) were evaluated in 288 unrelated individuals from the Ribeirão Preto area, São Paulo State, Brazil. DNA was extracted by salting-out and SNPs were genotyped by PCR-RFLP or Allele-Specific PCR, followed by 10% Polyacrylamide Gel Electrophoresis colored by silver staining.

Alleles and genotypes from 7 SNPs (rs181832, rs26722, rs2287949, rs250417, rs16891982, rs40132 and rs35394) presented statistically significant associations with at least two of the four considered morphological features (presence of freckles and eye, hair and skin pigmentation). For instance, alleles rs181832*T, rs2287949*G, rs250417*G and 374Phe (rs16891982) were observed in association with fair pigmentation features (like pale skin, green/blue eyes, and/or blond hair), while alleles rs181832*C, rs2287949*A, rs250417*C and 374Leu are associated with dark pigmentation. The associations from the 3 remaining SNPs deviate from this pattern. The present results corroborate previous findings and emphasize the role played by SNPs at SLC45A2 in the determination of pigmentation aspects of human populations.

Developmental validation of the HIrisPlex System: DNA-based eye and hair colour prediction for forensic usage

S. Walsh[1], L. Chaitanya[1], L.Clarisse[2], L.Wirken[3], J. Draus-Barini[4], L. Kovatsi[5], T. Sijen[2], P. de Knijff[3], W. Branicki[4,6] and M. Kayser[1]

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

[2]Department of Human Biological Traces, Netherlands Forensic Institute, The Hague, The Netherlands

[3]Forensic Laboratory for DNA Research, Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

[4]Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland

[5]Laboratory of Forensic Medicine and Toxicology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

[6] Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Kraków, Poland

Forensic DNA Phenotyping or 'DNA intelligence' systems are expected to aid police investigations by providing appearance information on unknown individuals when conventional DNA profiling or other means of investigations are non informative. Recently, we introduced the HIrisPlex system [1], capable of predicting both eye and hair colour including shade from DNA, which provide prediction accuracies of >94% for eye colour and >79% for hair colour on average [1]. Here we carried out a developmental validation study of the HIrisPlex assay following the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. We obtained complete 24 SNP profiles down to 63 pg of DNA. Species testing revealed human specificity for a complete SNP profile. Rigorous testing of simulated casework samples such as blood, semen, saliva, hair, and trace DNA samples, including extremely low quantity touch DNA samples, produced full SNP profiles. Concordance testing performed between five independent laboratories displayed consistent reproducible results on varying types of samples. Due to its design, the assay caters for highly degraded DNA samples and its robustness has already been demonstrated on modern and several hundred year old human remains [2]. By demonstrating that the HIrisPlex assay is fully compatible with the SWGDAM guidelines, we provide the first validated DNA test system for combined eye and hair colour prediction now available for immediate application in forensic laboratories to aid police investigation.

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The famous astronomer Nicolaus Copernicus likely had blue eyes and dark blond hair as revealed by model-based DNA prediction using the HIrisPlex system.

J. Draus-Barini[1], T. Kupiec[1], S. Walsh[2], M. Kayser[2], W. Bogdanowicz[3], W. Branicki[1,4]

[1]Section of Forensic Genetics, Institute of Forensic Research, Kraków, Poland

[2]Department of Forensic Molecular Biology, Erasmus MC University Medical Centre Rotterdam, Rotterdam, The Netherlands

[3]Museum & Institute of Zoology PAS, Wilcza 64, Warszawa, Poland

[4]*Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Kraków, Poland*

The skeleton of the famous astronomer Nicolaus Copernicus (1473-1543) was discovered in the Frombork Cathedral in Poland in 2005 and was successfully identified based on mitochondrial DNA evidence in 2009 [1]. The examined teeth also contained good quality nuclear DNA, which previously allowed the successful analysis of the *HERC2* rs12913832 polymorphism representing the most powerful single eye colour DNA predictor available. It was shown that Copernicus' DNA carried the homozygote rs12913832-C genotype [1], which only exists in light eyed individuals [2]. Various historical paintings however show Copernicus with different pigmentation traits. To estimate Copernicus' eye and hair color from his DNA we used the recently developed HIrisPlex system [3] that targets 24 eye and hair colour informative DNA variants and performed model-based predictions. We obtained a blue eye colour probability of 0.95 with a prediction accuracy of 99%. Hair colour was concluded using the previously developed prediction guide [3] as being dark blond based on the following probability values obtained: blond 0.774, brown 0.158, black 0.041, red 0.027 and a probability for light shade of 0.943. The accuracy of blond hair colour prediction was 69.5%. Our results highlight that Copernicus most likely had blue eyes and dark blond hair.

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Association of Melanocortin-1-receptor Gene Polymorphisms with Freckles in Chinese Han Population

J.Yan[1], L.P.Cao[1], Y.Ye[1], J.Wu[1], X.D.Fu[1], Y.P.Hou[1]

[1]*Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China*

The DNA-based prediction of physical traits has gained tremendous interest in criminal investigation. The association between genetic variations and external visible characteristics has become the topic of extensive study in forensic genetics. Several studies indicated that the melanocortin-1-receptor (MC1R) gene was a main determinant of human freckles. In this study, we aimed to examine the associations between the MC1R gene polymorphisms and freckles in Chinese Han population. Sequencing of the complete MC1R gene was performed on 20 random individuals from Chinese Han population. 12 single nucleotide polymorphisms (SNP) in the gene were found according to the sequencing results. The SNP markers were genotyped in 111 individuals with freckles and 124 controls by pyrosequencing (PSQ) and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The comparison of the genotypes and allele frequencies between freckled individuals and controls was done using the x^2 test. Our results showed that there was no association between the MC1R gene polymorphisms and human freckles. Further studies with a larger sample size are still needed to explore the association between the gene and freckles in Chinese Han population. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK16B01) and the Specialized Research Fund for the Doctoral Program of Higher Education, China (200806101086).

Theme 10. Body Fluid identification and mRNA testing

Identification of sperm-specific DNA methylation markers using bisulfite pyrosequencing

A. Vidaki[1,2], B. Daniel[1], D. Syndercombe Court[1,2]

[1]*Department of Forensic and Analytical Science, King's College London, London, UK*

[2]*Academic Haematology, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK*

Body fluid analysis does not only aid potential identification of the donor, but also can help reconstruct the sequence of events. Determining whether the biological material originates from semen may be very informative in sexual assault cases. Current confirmatory testing, such as microscopic identification of sperm heads or mRNA profiling, is often labour-intensive and suffers limitations. Here we propose a DNA-based assay, which enables identification of sperm DNA based on the detection of sperm-specific DNA methylation patterns in a series of genomic loci. Following genome-wide analysis using methylated DNA immunoprecipitation, a set of differentially methylated markers were identified. As methylation modification occurs only at cytosines followed by guanines (CpG sites), we measured the methylation levels of several CpG sites spanning each marker (LAT2, VAMP8, SLC25A31, C12orf12). DNA samples obtained from blood, semen, saliva and buccal swabs were collected and treated with sodium bisulfite, where only unmethylated cytosines are converted to uracil. The methylation levels were verified by bisulfite pyrosequencing and further quantified using dedicated software. Significant inter-individual variation was observed for selected loci; however, sperm could be successfully identified using 17 identified CpG sites. The assay was highly sensitive with expected methylation profiles being seen when using as low as 50pg of starting DNA material. Validation experiments using old, degraded as well as mock-casework samples were also performed. The results of this study demonstrate the applicability of epigenetic markers as a novel tool for body fluid identification and could be of substantial forensic significance.

Potential age determination using age-associated DNA methylation markers in blood

A. Vidaki[1], B. Daniel[1], D. Syndercombe Court[1]

[1]*Department of Forensic and Analytical Science, King's College London, London, UK*

Estimating the age of the donor from a bloodstain would be very beneficial in police investigations as it could reduce the number of suspects. A decline of gene regulation occurs over time; DNA methylation patterns have been proven to modify with age and have shown to be associated with age-related diseases. Hannum *et al.* (2013) identified 71 age-associated CpG sites and built a model for age estimation (error ±3.9 years). We selected 10 of the most promising markers and verified methylation patterns using bisulfite sequencing in blood samples. After analysing a range of DNA samples from different age groups we could predict age, although with significant error. The application of these markers across a range of tissues will also be investigated.

Evidence based strategy for normalization of quantitative PCR data in forensic miRNA analysis

C. Courts[1], E. Sauer[1], B. Madea[1]

[1]Institute of Legal Medicine, University of Bonn, Bonn, Germany

Micro-RNA (miRNA) based analysis of body-fluids and composition of complex crime stains has recently been introduced as potential and powerful tool to forensic genetics. Analysis of miRNA analysis has several advantages over mRNA but reliable miRNA detection and quantification using quantitative PCR requires a solid and forensically relevant normalization strategy.

In our study we evaluated a panel of carefully selected reference genes for their suitability as endogenous controls in miRNA qPCR normalization in forensically relevant settings. We analyzed assay performances and variances in venous blood, semen, menstrual blood, saliva and vaginal secretion and mixtures thereof utilizing highly standardized protocols, contemporary methodologies and computational algorithsms.

Based on these empirical results, we can recommend normalization to signatures of reference genes with the most stable expression level and the least expected variation.

To account for the lack of consensus on how best to perform and interpret quantitative PCR experiments, our entire study's documentation is according to MIQE guidelines (DOI: 10.1373/clinchem.2008), defining the "minimum information for publication of quantitative real-time PCR experiments".

Assessment of DNA profilability from putrefied bodies based on a newly developed quantitative grading system for putrefaction

C. Courts[1], Y. Hofmann[1], B. Madea[1]

[1] Institute of Legal Medicine, University of Bonn, Bonn, Germany

DNA-based identification of otherwise unrecognizable putrefied bodies is a common task in forensic genetics. However, with ongoing putrefaction DNA integrity will continuously decrease up to a point at which analysis is no longer possible. When this point is reached can as yet only be guessed because a systematic empirical investigation of the association of DNA profilability with different signs of putrefaction as well as the type of tissue used for DNA extraction is lacking so far.

In our study, for a collective of 75 putrefied bodies, putrefaction was rated using a newly developed 12 tier binary quantitative system to differentially grade putrefaction in decaying bodies. We then analyzed DNA profilability from samples of lung, aorta, liver, kidney, brain, and muscle tissue. Next, we screened the extent of DNA fragmentation in all samples using a self-devised pentaplex PCR. Then, DNA profilability by STR multiplex PCR was assessed for selected cases. In a final step DNA profilability was correlated to the putrefaction grading score and a model to predict STR typing success for putrefied bodies is proposed.

Validation of molecular marker for predicting the chronological age of a person

D. Zubakov[1], F. Liu[1], I. Kokmeijer[1], L. Broer[2], C.M van Duijn[2], M. Kayser[1]

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

[2] Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

Estimating the chronological age of a person from biological materials such as bloodstains that possess no morphological age information can facilitate the identification of unknown persons in the absence of body or bone/dental remains. Several molecular markers previously proposed for forensic age prediction were found to be practically not useful or their validation studies are still missing. Aiming to identify suitable candidate markers for chronological age prediction from blood, we used newly discovered mRNA and methylation DNA (methCpGs) markers obtained from genome-wide microarray screens, together with known age biomarkers (such as sjTREC, some methCpGs, and telomere repeat length) in blood samples from >200 individuals between 4 and 82 years of age. Multinomial regression analysis performed to estimate the age prediction effect for single and combined markers revealed the following ranking order of marker types with decreasing prediction value: methCpGs, sjTREC, mRNAs, and telomere repeat length. An age prediction model based on methCpG markers alone performed almost as good as the combined model with all markers tested. The best single methCpG marker in our set predicts chronological age with 81.2% accuracy (SE ±8.7 years), while the best 4 methCpGs together increase the prediction accuracy to 89% (SE ±6.5 years). Maximal possible prediction accuracy in this sample set was 91.5% (SE ±5.6 years) achieved with a combination of 16 methCpGs. Our results not only illustrate the power of molecular age prediction in general, but moreover indicate that particular DNA methylation markers can predict a person's age from blood with very high accuracy.

Capillary electrophoretic analysis of body fluid specific microRNA markers

G.A.Williams[1], M.L. Uchimoto[1], N. Coult[1], D. World[1] and E. Beasley[1]

[1]School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom

The characterisation of RNA molecules for the purpose of body fluid identification is currently a major field in forensic genetics; with a great deal of effort going towards the analysis of messenger RNA (mRNA). There is some focus on microRNA (miRNA) which is a more stable RNA molecule than mRNA; due to its short size and role in RNA interference. Most research into forensic miRNA analysis is based around quantitative PCR (qPCR) analysis. No substantial research has yet been carried out on capillary electrophoretic (CE) analysis of miRNA. Thus the aim of this study was to explore the viability of CE of miRNA markers and conduct comparisons with qPCR.

Samples of blood, saliva, semen, vaginal material and skin cells were obtained from a variety of volunteers with their informed consent. All samples then underwent standard DNA extraction using QIAAmp DNA mini kit; using the buccal swab protocol regardless of body fluid type. cDNA synthesis was carried out using stem-loop reverse transcription using commercially available stem-loop primers. qPCR was carried out using a 7500 Fast Real-Time PCR Machine and using commercially available miRNA assays. The amplified product then underwent fragment analysis using an ABI 3130 genetic analyser.

The findings have demonstrated that CE analysis of miRNA markers could be viable for the purpose of forensic genetics. The fragment sizes (between 50 and 100 bp) suggest that such CE based miRNA assays could be multiplexed with STR kits with minimal modifications; thus enhancing the capability of DNA profiling facilities.

Body fluid mixtures; resolution using forensic microRNA analysis

G.A.Williams[1], M.L. Uchimoto[1], N. Coult[1], D. World[1], E. Beasley[1]

[1]School of Applied Sciences, University of Huddersfield, Huddersfield, United Kingdom

Body fluid identification is a crucial aspect of forensic biology; particularly in sexual assaults which is usually characterised as a mixture of body fluids. Whilst there are considerable efforts to identify body fluids using genetic markers; no substantial research appears to have been carried out on mixed body fluids. This is a potentially complex area and before such genetic based body fluid identification can be utilised, an understanding of the effects of mixtures on the results is required. Can body fluid mixtures be identified and if so, what is the value of the information gained?

Samples of blood and saliva were acquired from volunteers with informed consent. The samples underwent total RNA extraction. A range of mixtures were then prepared in the mixing ratios of 1:1, 2:1, 5:1, 10:1 and 20:1 (both with blood and then with saliva as the major contributor). Single source controls were included. All samples then underwent stem-loop reverse transcription and quantitative PCR analysis targeting blood and saliva specific microRNA markers using commercially available kits.
When compared with the single source controls, the mixed body fluid samples could be easily identified. By comparing the samples with the 1:1 blood/saliva mixture, the major and minor contributor for each body fluid mixture could be correctly identified. Finally, when compared with the mixed DNA results, the major body fluid could be correctly associated with the major DNA contributor and vice versa.

Evaluation of PSA-test on anal swabs from male dead bodies.

G. Schmit[1], D. Hoton[2], S. Adamis[3], F. Bonbled[1]

[1]Médecine Légale, Cliniques Universitaires St-Luc, Brussels, Belgium

[2]Anatomie Pathologique, Cliniques Universitaires St-Luc, Brussels, Belgium

[3]Unité d'expertise génétique GENEX, Brussels, Belgium

Introduction and aims: we've been confronted to a specific case in which, after having discovered a putrefied male dead body, we realised an anal swab because of a context of possible sexual assault. PSA-test was positive but genetic profile was only the one of the victim.

Materials and method: we made anal swabs on 40 male corpses, with different post-mortem delays. For each case we made 3 anal swabs : one for PSA-test, one for research of sperm cell, one for genetic profile. We also made a blood swab as a reference. For half of them, we also collected a prostatic sample for histological examination to see if a correlation exists between the autolysis degree of the prostatic tissu and the presence of PSA (semen) in the anus.

Results and discussion: First results showed that PSA-test was almost always positive, whatever was the post-mortem delay. Only the genetic profile of the victim has been found. PSA-test shouldn't be used as an indicative test in case of presumed male sexual assaults on samples coming from dead bodies.

Evaluation of mRNA specific markers using a pentaplex system for the identification of skin and saliva from contact trace evidence

I. Gomes[1], B. Strohbücker[1], M.A. Rothschild[1], P.M. Schneider[1]

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

A vast number of mRNA markers have been proposed for the identification of the source of biological stains recovered from crime scenes. Blood, semen, saliva, menstrual blood, vaginal secretions, urine and skin represent the majority of sample types in body fluid identification. However, certain sample types, such as blood, may not be considered as main targets for identification in comparison to "non-coloured" and contact trace evidence which are even more frequently found at crime scenes, given the high sensitivity of the currently available STR typing kits. Skin and saliva are examples of such evidentiary material and are often present as single sources or mixture, representing a greater challenge for identification. Therefore, the need for highly specific and sensitive markers is essential. In this work, a pentaplex mRNA identification system was developed and optimized for the analysis of three skin genes LCE1C, LOR and CDSN and two saliva genes, HTN3 and STATH. The specificity of the selected mRNA transcripts was evaluated through the analysis of saliva and skin mixtures and by cross-reactivity testing. In addition, sensitivity was also analysed by testing common objects of contact trace evidence such as telephone mouthpiece, keyboards, bottles, cups, computer mouse, pens, etc. Results demonstrated LCE1C as the most sensitive skin mRNA marker tested in mock forensic samples. On the other hand, no saliva markers were detected in the same sample set, demonstrating the absence of cross-reactivity. The five skin and saliva mRNA markers revealed to be highly specific and useful to distinguish between skin and saliva.

Issues Involved with mRNA Body Fluid Identification in a Casework Setting

J Stacey[1], N Curnow[1], S Dixon[1], H Baker[1], M Fallow[1], R Fleming[1], P Simon[1], R Wivell[1], SA Harbison[1].

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

At ESR we have developed a multiplex reverse transcriptase (RT-PCR) PCR system known as CellTyper that utilises messenger RNA (mRNA) for the identification of blood, saliva, semen, menstrual blood and vaginal material in individual stains or in mixtures of body fluids. This multiplex can detect both sperm and seminal fluid (semen without spermatozoa present).

By co-isolating the RNA and DNA from the same sample and using the CellTyper multiplex, we can determine the type of body fluid present while also generating a DNA profile from the same stain.

Here we undertake an assessment of issues we have encountered since CellTyper was implemented into our casework laboratory. This includes analysis of their impact on casework and possible actions that can be taken to reduce or eliminate their effect on the capability of the system.

DNA methylation profiling for forensic body fluid identification

J.H. Kim[1,3*], J.L. Park[2,3], S.Y. Kim[2,3], Y.K. Choi[4], S.H. Oh[4], Y.S. Kim[2,3]

[1]Biomedical Proteomics Research Center,

[2]Medical Genomics Research Center, KRIBB,

[3]Department of Functional Genomics, University of Science of Technology, 111 Gwahang-no, Yuseong-gu, Daejeon, Republic of Korea,

[4]DNA Forensic Division, Supreme Prosecutor's Office, 157 Banpodae-ro, Seocho-gu, Seoul, Republic of Korea

Analysis of DNA sequence from crime scenes provides crucial information on the identity of individuals, but does not provide information on the type and origin of the biological material. Recently, many genome-wide DNA methylation profiling studies have shown that DNA methylation is tissue or cell type specific, suggesting a potential of DNA methylation for body fluid identification. Here, we used Illumina's Human Methylation 450K array to identify body fluid specific DNA methylation makers among blood, saliva and vaginal fluids. From 8 samples (3 blood, 2 saliva, and 2 vaginal fluids) profiled, we selected several hundred specific hypermethylated CpG sites for each tissue. We then validated three of them (chr17:80834089, chr10:8085349 and chr2:242974096) in 21 independent body fluid samples by pyrosequencing. Receiver Operator Characteristics (ROC) analysis showed that each of the three markers has an AUC value of 1, suggesting that they can be useful markers for body fluid discrimination. We are now performing further array experiments with additional samples including semen samples and validating the usefulness of DNA methylation markers in a large number of body fluid samples. In conclusion, analysis of DNA methylation is a promising approach for forensic body fluid identification.

mRNA and DNA profiling of minute body fluid stains

J. Vella[1,2], I. Quinones-Garcia[1], A. Bell[3], B. Daniel[1]

[1]Kings College, London, UK

[2]University of Malta, Malta

[3]Metropolitan Police Service, London, UK

Co-extraction of nucleic acids from a body fluid stain would serve to identify the cellular origin of the body fluid through messenger RNA (mRNA) profiling and the individual from whom it came through DNA analysis.

A reverse transcription endpoint polymerase chain reaction (PCR) method was optimised to identify blood, semen and saliva stains by measuring expression levels of haemoglobin beta (HBB), protamine 1 (PRM1) and statherin (STATH). DNA and RNA were co-extracted from five 5µl and 1µl stains for each body fluid type.

HBB was expressed in all blood stains. PRM1 was expressed in 80% of the semen stains. STATH was expressed in: all 5µl saliva stains and 20% of the 1µl samples. Full DNA profiles were obtained for 5µl blood and 5µl and 1µl semen stains. Partial DNA profiles were obtained for 5µl saliva stains. No DNA profiles were obtained for 1µl blood and saliva stains.

This research shows how it is possible to recover both types of nucleic acids from blood and semen samples though further study is required for saliva samples. The study calls for a validation of: the specificity and sensitivity of the mRNA markers; and the sensitivity and reproducibility of the method.

Genome-wide miRNA profiling for forensic body fluid identification

S.Y. Kim[1,2], J.H. Kim[1], J.L. Park[1,2], S.M. Park[1,2], Y.S. Kim[1,2], H.C. Lee[3], K.M. Woo[3]

[1]Medical Genomics Research Center, KRIBB,

[2]Department of Functional Genomics, University of Science of Technology, 111 Gwahang-no, Yuseong-gu, Daejeon, Republic of Korea,

[3]DNA Forensic Division, Supreme Prosecutor's Office, 157 Banpodae-ro, Seocho-gu, Seoul, Republic of Korea

In forensic science, identifying body fluids found at a crime scene provide much information in many cases. Despite their usefulness, current enzymatic, immunological and mRNA tests have limits such as low specificity, lack of sensitivity, and instability. In this regard, miRNAs are promising as they are present in human body fluids in a remarkably stable form protected from endogenous RNase. In this study, we performed miRNA microarray experiment with 20 Korean body fluid samples (blood, saliva, semen, and vaginal fluids) to explore the usefulness of miRNAs for body fluid identification. Sixteen body-fluid-specific candidate miRNA were selected by calculating Shannon entropy and Q-values for each miRNA. To validate the reliability of the 16 candidate miRNA in other datasets, we collected miRNA expression datasets (blood, saliva, semen) publicly available from the Gene Expression Omnibus. The miRNA markers from our data showed body fluid-specific expression pattern in public datasets, validating the usefulness of the selected 16 markers. We are now confirming the 16 miRNA markers in 96 Korean body fluid samples by using Nanostring technology. In conclusion, miRNAs are promising molecular markers for forensic body fluid identification.

A one year time course study of human RNA degradation in body fluids under dry and humid environmental conditions

M. Sirker[1], I. Gomes[1], M.A. Rothschild[1], P.M. Schneider[1]

[1]Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

Human body fluids such as blood, saliva and semen are some of the forensically most relevant stains found at crime scenes. Hence it is crucial to have appropriate methods to identify the biological origin of evidentiary traces, such as mRNA profiling. Therefore, a stable set of markers and the knowledge about the effects of RNA degradation under different environmental conditions is essential. The aim of the current work was to compare RNA degradation for human blood, semen and saliva at three different concentrations during a one year period and exposed to dry and humid conditions. Also, the efficiency of two different RNA extraction methods was compared. Therefore, 5-, 0.5- and 0.05-µL-sized stains were prepared in triplicates for all three samples types and stored at room temperature exposed to day light and dry and humid conditions. Total RNA was extracted every two months with a column-based DNA/RNA co-extraction kit and with an automated silica-based RNA purification method. Downstream mRNA analysis was conducted for the following tissue-specific genes: for blood, HBB and SPTB; for saliva, STATH and HTN3 and for semen, PRM1 and PRM2. Reverse transcription endpoint PCR was performed in duplicate followed by CE detection. Results demonstrated that mRNA can be recovered in sufficient quantity by means of both methods. Nevertheless, the column-based method seems to be more suitable to recover mRNA from the humidity degraded samples. Our results provided useful information for the selection of stable and sensitive mRNA markers, in particular, when samples are recovered after long environmental exposure.

A validation study of mRNA markers for skin cell identification

S.E. Hall[1,2], R.A.H van Oorschot[1], R.J. Mitchell[2], K.N. Ballantyne[1]

[1]Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Victoria, Australia

[2]Department of Genetics, La Trobe University, Bundoora, Victoria, Australia

At present, there is a strong trend in forensic genetics research for the development of alternative approaches in identifying the cellular origin of biological stains from crime scene samples. Many laboratories are focused on the identification of tissue-specific messenger RNA (mRNA) markers for the development of an assay to detect forensically relevant human body fluids. Whilst skin-specific mRNA transcripts have been described previously [1], variation within and between individuals has been observed, thus complicating the determination of the presence of skin using these markers. The objectives of this study were to develop and optimize a multiplex of three skin specific gene markers; loricrin (LOR), corneodesmosin (CDSN) and keratin 9 (KRT9)and 1 house-keeping marker, ß-Actin (ACTB) using an endpoint PCR assay to analyse expression data from a range of relevant samples. Marker specificity and suitability were evaluated for their inclusion in future forensic casework.

The presence of the three skin mRNA markers has been successfully confirmed from swabs of human skin obtained from 20 individuals in each of 6 different body sites (forehead, neck, arm, palm, leg and sole). Significant variation was observed in the relative expression of the three genes across the body sites, with some individuals consistently failing to express one or more of the targets. Inter-individual variation was also evident. Accordingly, these markers must be used with caution in the identification of skin in forensic samples.

Visser et al. Int J Legal Med 125 (2011) 253-263.

A new strategy for body source identification of tumor sample

S.M. Zhao[1], S.H. Zhang[1], N. Zhang[1], K. Sun[1], T.Z. Que[1], Z.M. Zhao[1], C.T. Li[1]

[1] Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Sciences, Ministry of Justice, P.R. China, Shanghai, P.R. China

Body source identification of tumor sample with likelihood ratio is usually difficult because of the high mutation rate of STR in tumor. Here, we developed a new strategy to identify the body source of tumor sample. In this study, 423 tumor and their homogenous normal samples were genotyped with Goldeneye 20A multiplex STR genotyping system, which was including 19 STR loci widely used in forensic casework. An improved identity by state (IBS) scoring system was adopted to calculate the *IBS* score, the number of loci with 2 alleles (A_2), 1 allele (A_1) or 0 allele (A_0) sharing within each tumor-normal pair (TNP). The binomial distribution of the four variates in unrelated individual pairs (UIP), parent-offspring pairs (TOP) and full-sibling pairs (FSP) were derived based on the allele frequencies of the 19 STR loci in Chinese Han. The results showed that 30.97% of tumor samples carried one or more STR loci with genotypic alteration. According to the binomial distribution of the four variates in the studied groups, A_2 was most effective to distinct TNP from UIP, TOP, or FSP. Using the criterion of $A_2 \geq 15$, the body source of 98.82% of tumor samples was successfully determined. Contrarily, the probability of $A_2 \geq 15$ in UIP, TOP and FSP was 0.00%, 0.00% and 0.02%, respectively. We further demonstrated that the strategy of A_2 binomial distribution was more powerful than likelihood ratio method in body source identification of tumor sample. This research was supported by Nature Science Foundation of Shanghai (11ZR1438400).

5 miRNA expression analyze in post-mortem interval (PMI) within 48 hours

Hui Wang[1], Jiong Mao[1], Yingbi Li[2], Haibo Luo[2], Jin Wu[2], Miao Liao[2], Weibo Liang[2], Lin Zhang[2]

[1] Crimial Investigation Department of Chengdu Public Security Bureau, Chengdu, PR China

[2] Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, PR China

MicroRNAs (miRNAs) are small, noncoding RNA molecules with an important role in regulating gene expression at the posttranscriptional level. They present interesting characteristics in forensic science, such as bodyfluid-special expression, tissue-special expression and stability at post-mortem. We purposed a preliminary study on mice, to analyzing the 5 miRNAs expression pattern in PMI within 48 hours.

33 adult C57 male mice were randomly grouped into 10 PMI time points within 48 hours under the room temperature and 0h (just after death), with 3 mice in each group. The cardiac muscle, liver, brain and skeletal muscle were collected from each mouse and stored at -80 . Total RNA was extracted using an RNAiso Reagent (Takara, Japan), following RT using Takara RNA PCR kit (AMV) (TAKARA, Japan) and SYBR assay of reatime PCR using SYBR® Premix Ex TaqTM Kit (Fermentas, Canada). After the results were normalized against U6B, we found that in the first 24 hours of PMI, all 5 target miRNA from liver tissue did not show any correlation, but between 24 to 48 hours, 3 miRNAs decreased obviously. The result shows that miRNAs present good stability in the first 24 hours of PMI, and some of them begin to decrease after 24 hours. Therefore, before we study on the miRNA of PMI, the miRNA degradation patterns should be analized at first.

Micro RNA profiling for the detection and differentiation of body fluids in forensic stain analysis

Peng Bai[1], Weibo Liang[1], Wei Deng[1], Li Wang[1], Bing Long[1], Kuanlin Liu, Lin Zhang[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu, PR China

[2]Traffic Administration Bureau, Chengdu Public Security Bureau, Chengdu, China

Micro-RNAs (miRNAs) are a class of small non-coding RNA (ncRNA) molecules with a length of 18 to 24 nucleotides which play an essential regulative role for many cellular processes. In past few years, some researches show interests of body fluid/tissue identification through miRNA profiling.

We collected four kinds of body fluid samples which are the stains of blood, semen, saliva and vaginal secretions. A panel of eight miRNA markers which are miR-451, miR-16, miR-135b, miR-10b, miR-658, miR-205, miR-124 and miR-372 were selected to be analyzed through SYBR qPCR. All the data was normalized against U6B. We analyzed through two-dimensional miRNA assays and also developed four N-J trees for different kinds of body fluids which can be used to identify unknown body fluid. Compared with other researches, our results shows no difference with Hanson's and Madea.B's researches which using SYBR method, but are not as same as Zubakov and Hou's researches which using the Taqman method. Maybe the reason of these differences is the much higher sensitivity and specificity of Taqman assay. Even though our result of research shows good reproducibility of the SYBR assay.

Theme 11. Evaluation of DNA evidence

Is he or is he not the father? Pros and Cons effect of following standardized interpretations

A.El Andari[1], I. Mansour[1]

[1]Faculty of Health Sciences, American University of Science and Technology, Beirut, Lebanon

Parentage testing determines whether or not a particular individual is the biological parent of a given child. It provides a numerical proof of the likelihood of an alleged parent to be the biological parent of this child. If so, the child should share at least half of the DNA profile alleles with each of the biological parents. Afterwards, statistical calculations are performed using the likelihood ratio approach to test the hypothesis that an alleged parent is the biological parent against the hypothesis that an unknown individual, from the same ethnic background, is the biological parent. For that, a given number or STR systems are evaluated and allele frequencies for that ethnic background are used.

Lebanon lacks legislation concerning parentage testing; therefore Lebanese relationship testing laboratories usually follow international standards as to the cut off limit for parentage positivity interpretations. This could differ among countries, laboratories and experts. Besides, Lebanon has relative high consanguineous marriage rates (ranging from 32% up to 44%), whereby individuals involved in the parentage dispute may share alleles due to endogamy and consanguinity effect. Consequently; the potential of uncertainties in the interpretation of parentage disputes increases if remedial corrections for such sub populations are not incorporated, as it is frequently the case in local practices. In addition, omitting a unique mismatch in inconclusive parentage testing and reporting the POP obtained after that analysis is also a common practice among Lebanese relationship testing laboratories.

As part of ongoing research studies tackling uncertainties in consanguineous and endogamous populations in relationship testing, twelve inconclusive cases were evaluated. A simulation of DUO versus TRIO analysis, along with different profile sizes ranging from 12 up to 24 STR markers was performed in order to assess the different possible parentage dispute conclusions.

Results shifted from positive parentage conclusions to non-parenthood when increasing STR markers from 12 to 16 and/or 24, when of using DUO versus TRIO analysis and when mutation rates were incorporation to the analysis. Also, inconclusive cases, presenting one mismatch were cleared by using additional STR systems and showed unambiguous exclusions. On the contrary, some Trio families showing a clear paternity exclusion showed a complete inclusion when simulating Duo families.

These results will be used to gauge proper corrections by mathematical procedures and to recommend appropriate profile size as to the number of genetic systems in the Lebanese populations and, probably, to similar populations with endogamy or consanguinity. Similar conclusions were also reached by different studies conducted in our laboratory, where the effect of consanguinity and endogamous marriages in the Lebanese population was evaluated. This work was granted with the necessary multiplex kits by Promega Corporation.

Snipper 2.0: Enhancements to an online forensic classifier

A. Gómez-Tato[1], J.A. Álvarez-Dios[1], M.A. Casares de Cal[1], C. Phillips[2], C. Santos[2], M.V. Lareu[2]

[1] Faculty of Mathematics, University of Santiago de Compostela, Spain.

[2] Forensic Genetics Unit, University of Santiago de Compostela, Spain.

We outline enhancements to a widely used online forensic classifier: Snipper 2.0 (http://mathgene.usc.es/snipper/): a portal for classifying forensic SNP, Indel or STR profiles compared to classes that are defined by fixed or user-uploaded training sets of marker variability in different populations, or in the case of phenotype classifications, sets of physical characteristics. Classifications are based on a naïve Bayesian approach so that the likelihoods of membership to each population or phenotype class are calculated and the quotient of likelihoods measures the classification strength. Training sets can comprise allele frequencies (applicable to STRs and linked markers requiring haplotype counts) as well as genotype profiles (applicable to unlinked SNPs and Indels).

Among the new characteristics we describe are: improved graphical output, new pre-defined training sets for commonly used forensic SNP sets that encompass all five major population groups, a refined classification algorithm and additional functionality related to the detection and analysis of population admixture.

STRmix: The application of a continuous model expert system to forensic casework in New Zealand

C McGovern[1], D Bushell[1], J-A Bright[1]

[1]ESR Ltd, Auckland, New Zealand

STRmix is software-based expert system that applies a fully continuous approach to DNA profile interpretation. ESR implemented STRmix into routine forensic casework in August 2012, for use with both Identifiler™ and MiniFiler™ DNA profiling data. This presentation describes our laboratory's experience with the software during its first year of case data. The improved interpretative capability and advanced database search functionality are both illustrated with examples of challenging profiles. With appropriate care, low level, complex mixed DNA profiling data can be effectively and objectively assessed. Meaningful database searches can be conducted on mixed DNA profiles previously considered 'unresolvable', even when individual contributors may be related.

Optimized mass fatalities victim identification: an airplane crash as a test case.

D. Corach [1,2], A. Sala[1,2], C. Bobillo[1,2], M.Caputo[1,2], E. Alechine[1,2], M. Irisarri[1], M.Marino[3], E. Canónaco[4], C. Rodriguez[4].

[1]Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina.

[2]CONICET (National Scientific and Technical Research Council).

[3]Laboratorio de ADN Poder Judicial de Mendoza, Argentina

[4]Cuerpo Médico Forense de la Justicia Nacional, Department of Justice, Argentina

Efficient corpse tissue preservation, automated DNA extraction, sensitive DNA quantization and expert analysis software makes possible to design highly efficient workflow that speed up the mass disaster victim identification process from highly fragmented human bodies. We describe the workflow employed in the reconstruction of the identity of 22 victims fragmented in 418 remains emerged from the airplane crash occurred on May 18, 2011 in Río Negro Province, Argentina. Fatal victims identification was performed by comparison with first degree relatives by autosomal and Y-STRs analysis. A SAAB plane carrying 19 passengers and three crew members (3 females and 19 males) exploded at high altitude over the soil level (approx. 2000m). All bodies were severely

fragmented and not complete skulls but a high number of isolated cranial bones were found. Remains were collected at the disaster area and sent to Buenos Aires where samples selection took place and preserved in 50 ml polypropylene tubes containing solid Sodium Chloride. DNA extractions from muscle and bone were performed using a semi-automated DNA purification system; quantization was carried out by real-time PCR using commercial kits and data analysis performed with the help of expert software. The implementation of this strategy allowed us to identify all the victims in one week. However, the complete task of identifying all remains recovered within the disaster area took over ten months due to wide dispersion of fragments and the court decision to identify each morphologically recognizable human tissue found within the disaster area.

An assessment of Bayesian and multinomial logistic regression classification systems to analyse admixed individuals

D. McNevin[1], C. Santos[2], A. Gómez-Tato[3], J. Álvarez-Dios[3], M. Casares de Cal[3], R. Daniel[4], C. Phillips[2], M.V. Lareu[2]

[1] *National Centre for Forensic Studies, University of Canberra, ACT, Australia.*

[2] *Forensic Genetics Unit, Institute of Legal Medicine, University of Santiago de Compostela, Spain.*

[3] *Faculty of Mathematics, University of Santiago de Compostela, Spain.*

[4] *Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Victoria, Australia.*

There are a number of valid approaches to classification of SNP profiles that have forensic relevance for ancestry analysis and phenotype prediction. The *Snipper* classifier uses a näive Bayesian approach that provides likelihood ratios and makes a classification based on the highest likelihood value. A more sophisticated Bayesian method is used in the *Structure* algorithm commonly applied to population genetic analysis. Multinomial logistic regression (MLR) is used to assign probabilities to discrete outcomes from categorical data such as genotypes.

A comparison of the Bayesian classification approaches of *Snipper* and *Structure* with MLR was made in order to assess the ability of each approach to detect and analyze admixed individuals, i.e those with detectable co-ancestry resulting from mixed parentage. Test populations comprised admixed 1000 Genomes populations: Mexicans (MXL); Colombians (CLM); Puerto Ricans (PUR); African Americans (ASW) and unadmixed 1000 genomes populations from Africa, Europe and East Asia. The reference populations comprised those of the HGDP-CEPH panel relevant to the test populations, i.e. African, European, East Asian and Native American.

The results of analyses made with each of the three classification systems are outlined. *Snipper* has now been adapted to include estimates of the admixture proportions for SNP profiles that show indications of co-ancestry.

Using MCMC to determine how much we don't know about what we don't know in DNA profiling

D. Taylor[1], J. Bright[2], J. Buckleton[2]

[1] *Forensic Science SA, Adelaide, South Australia*

[2] *ESR Ltd, Auckland, New Zealand*

A typical assessment of the strength of DNA evidence is based on allele frequencies, determined from a population database. Commonly a range is placed around any statistic generated, which takes into account the sampling variation inherent in compiling a database from a subset of a population.

Bayesian methods of dealing with database sampling variation produce a distribution for the statistic, from which the value at the desired interval is reported.

Population database sampling uncertainty represents only one, and arguably the smallest, of the sources of uncertainty that affect DNA statistical calculations. Potentially having a much larger effect on the statistic are:

- The uncertainty in the value of Fst used in calculations
- The uncertainty in the probabilities given to possible contributor genotypes
- The uncertainty in the number of contributors and
- The uncertainty in the composition of the pool of possible offenders

This study investigates the effect of each of the above mentioned sources of uncertainty on a DNA statistic (the likelihood ratio) distribution and demonstrates how these changing distributions would affect the reported statistic.

Analyses were carried out using MCMC analyses on DNA profiles that represent a range of complexity and strength. By taking all uncertainty into account in the generation of a credible interval statements such as "the LR is above X with 99% probability" can be given.

GenoProof Mixture 2.0 – An expert system for forensic sample and mixture interpretation

F. Götz[1]

[1] *Qualitype GmbH, Dresden, Germany*

The analysis of DNA samples, especially of mixtures, is one of the most challenging tasks in forensic labs. This software package has been developed to meet these challenges and simplify data interpretation on the basis of the experience of interpreting hundred thousands DNA samples of different quality.

The software makes use of new algorithms designed for raw data analysis of fsa and hid files including size calling and allele calling algorithms as well as new methods for artefact identification, among others. This technique has been validated with hundreds of problematic DNA samples that normally can not be interpreted by other software packages. These newly developed algorithms allow analyzing almost any sample file without any manual adjustments.

For the interpretation of mixed samples additional tools such as a direct comparison of different samples or a simulation of mixtures in silico were implemented.

GenoProof Mixture 2.0 provides a full spectrum of biostatistical mixture analysis in compliance with the guidelines of the ISFG. This includes the free formation of hypotheses and calculation of both RMNE and likelihood ratios. Furthermore, drop out and drop in probabilities can be considered.

The newly developed raw data interpretation combined with a full set of biostatical tools allows a fast and secure analysis of even difficult DNA samples in high throughput.

The distribution of likelihood ratios when contributors may be related

Guro Dørum[1], Thore Egeland[1,2] and the EUROFORGEN-NoE Consortium

[1]*Norwegian university of Life Sciences, Ås, Norway*

[2]*Norwegian institute of Public Health, Oslo, Norway*

When evaluating complex DNA profiles involving multiple contributors it may be difficult to assess the strength of the evidence based on the magnitude of the likelihood ratio (LR). A possible approach is to evaluate the LR with respect to likelihood ratios expected under the defense hypothesis. Consider e.g. the hypotheses H_p: victim + suspect vs. H_d: victim + unrelated unknown. The questioned profile, in this case the suspect, can in the LR model be replaced with random profiles reflecting the population allele frequencies, and the observed LR can be compared with the distribution of likelihood ratios for random men.

We have developed an algorithm that can compute the exact distribution of likelihood ratios based on all possible profiles that may occur under the defense hypothesis. From this it possible to find the exact probability of observing an LR of this magnitude if the defense hypothesis is true, which can be used as additional quantitative measure of the strength of the evidence.

The algorithm can handle hypotheses that specifies general family relationships between contributors, e.g. H_p: victim + suspect vs. H_d: victim + untyped first cousin of the suspect. The distribution of likelihood ratios under Hd must reflect that the untyped contributor is the first cousin of the typed suspect.. Disregarding a plausible close relative of the suspect as an alternative contributor may overestimate the evidence against a suspect. The methods and freely available software we present provide the tools needed to assess evidence involving related contributors.

Approaching variation: an attempt

Harald Niederstätter[1], Gabriela Huber[1], Walther Parson[1,2]

[1] *Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria*

[2] *Penn State Eberly College of Science, University Park, PA, USA*

Neutral genetic variation among individuals forms the substrate of forensic DNA profiling, a discipline where microsatellites are of major interest. Not least due to the rapid advances in the elucidation of the structure and sequence of the human genome, revealing a plethora of novel markers, other types of polymorphisms at single nucleotide positions (SNPs) or deletions/insertions (DIPs), gained significance in forensic science and beyond. Established applications, e.g. genotyping of Y-chromosomal SNPs and sequencing of the mitochondrial control region or parts thereof, fill vital niches, and novel approaches revealing bio-geographic ancestry or physical traits attract growing attention in the forensic scene.

However, none of the available genotyping methods perfectly meets all of the diverse needs in everyday genetic testing. Hence, a number of questions shape the decision for or against a particular approach:

- How many samples are to be analyzed, in which time, on what budget?
- How much sample do I have?
- How good/bad is the anticipated DNA quality?
- Do I expect mixed stains?
- Do I need to detect 100% of the variation or can I focus on (a) specific site(s)?
- Will I need multiplexed analyses?
- Which level of experimental complexity is tolerable?
- Which instrumentation is available?

With this in mind, we set out to provide a concise overview regarding the pros and cons of a number of homogeneous or non-homogeneous methods relying on allele-specific or non-allele-specific PCR amplification. These approaches either facilitate gene-scanning or pinpoint particular sites. Examples taken from our current research will be given where appropriate.

This work received financial support from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 285487.

Interpreting LCN DNA profiling results – biological versus statistical models

J. Patel[1], S. Vintiner[1], P. Simon[1], S. Cooper[1], L. Melia[1], C. McGovern[1], R. Wivell[1], J. Veth[1], L. Russell[1], J-A Bright[1].

[1]*Institute of Environmental Science and Research Limited (ESR), Auckland, New Zealand*

Low Copy Number (LCN) DNA profiling analyses use replicate amplifications and a consensus framework for reporting, often referred to as the biological model. In this model each individual replicate may provide general information about the DNA present in a sample, such as whether a mixture of DNA from more than one individual might be present. However, each individual DNA profiling result is required to be replicated before the result can be confirmed and used for comparison purposes.

STRmix™ is an expert system that utilises a fully continuous approach to DNA profile interpretation. The use of this expert system has been tested for interpreting results from LCN profiling analyses to determine if it is suitable. STRmix™ is referred to here as the statistical model.

Here we present the results from LCN samples that include mixtures of known contributors, which have been interpreted using both the biological model and statistical model.

Using MCMC to determine how much we don't know about what we don't know in DNA profiling

D. Taylor[1], J. Bright[2], J. Buckleton[2]

[1] *Forensic Science SA, Adelaide, South Australia*

[2] *ESR Ltd, Auckland, New Zealand*

A typical assessment of the strength of DNA evidence is based on allele frequencies, determined from a population database. Commonly a range is placed around any statistic generated, which takes into account the sampling variation inherent in compiling a database from a subset of a population.

Bayesian methods of dealing with database sampling variation produce a distribution for the statistic, from which the value at the desired interval is reported. Population database sampling uncertainty represents only one, and arguably the smallest, of the sources of uncertainty that affect DNA statistical calculations. Potentially having a much larger effect on the statistic are:

- The uncertainty in the value of Fst used in calculations
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This study investigates the effect of each of the above mentioned sources of uncertainty on a DNA statistic (the likelihood ratio) distribution and demonstrates how these changing distributions would affect the reported statistic.

Analyses were carried out using MCMC analyses on DNA profiles that represent a range of complexity and strength. By taking all uncertainty into account in the generation of a credible interval statements such as "the LR is above X with 99% probability" can be given.

Going totally Bayesian: Lab experiences when moving to a continuous DNA interpretation model

D. Taylor[1], J. Bright[2], J. Buckleton[2]

[1] Forensic Science SA, Adelaide, South Australia

[2] ESR Ltd, Auckland, New Zealand

In 2012 both Forensic Science SA in South Australia (FSSA) and The Institute for Environmental Science and Research in New Zealand began using a fully continuous MCMC based software system for DNA profile interpretation. Moving to such a system has a number of advantages, not the least of which is the ability to provide a statistical weighting for the comparison of any reference to virtually any evidence profile.

With the transition from human-based binary to computer-based continuous systems of DNA profile interpretation we set out to adopt an approach that extended the ethos of software into all other aspects of our interpretation, reporting and workflow. That is, one of being unbiased and objective.

Adopting a continuous approach has had far reaching effects, from the exhibits we accepted for analysis, the profiles we chose to analyse, the way we constructed our likelihood ratios, the way we reported the result and ultimately the way we provide evidence in court.

Competing pressures to complete objectivity were practicality, resources and comprehensibility of results. Large efforts were made to remove binary based interpretational terms such as 'not excluded', 'inconclusive' and 'match' but still present the result in a way that a jury and court room understand.

This presentation highlights some of the methodology adopted at FSSA in an effort to remove as much subjectivity as possible and the challenges faced in doing so.

Evaluation of mixed profiles from complex casework using the Forensim-LRmix module

A. Mosquera-Miguel[1], A. Freire-Aradas[1], M. Fondevila[1], A. Rodríguez[1], H. Haned[2], A.Carracedo[1], MV. Lareu[1].

[1] Unidade de Xenética Forense, Instituto de Ciencias Forenses "Luis Concheiro", Facultade de Medicina, Universidade de Santiago de Compostela, Spain.

[2] Netherlands Forensic Institute, The Hague, The Netherlands.

The interpretation of mixed DNA profiles is one of the principal current challenges in forensic genetics. A number of software are available to assist in the interpretation of complex mixtures. In this study, we evaluate, with real, complex cases, Forensim: the first publicly available open-source tool dedicated to forensic DNA evidence interpretation. The LRmix module of the Forensim package (Haned, 2011) offers biostatistical tools that facilitate complex profile analysis.

We collected all the cases involving mixtures received at our Institute in the past three years. This mainly involves sexual assault cases, with one or multiple alleged aggressors, and nail scraping samples, typical of a victim's self-defence response. For a proportion of cases described here, the number of possible contributors was more than three. Our main goal was to evaluate the LRmix module in casework, where both the variability of mixture scenarios and profile complexity require flexible and high standards interpretative tools.

We analyzed each mixed profile with LRmix and performed an evaluation of the obtained results. We tested various hypotheses, when applicable, adding several replicates and including various contributor combinations. We compared the results using LRmix with the original reports. The advantages and disadvantages of routine use of LRmix are discussed. Our experience suggest that LRmix provides an informative tool to explore various mixture scenarios, however, expert profile scrutiny continues to be a key part of the interpretation of complex profiles.

Acknowledgements: AMM and MF are supported by the European Forensic Genetics Network of Excellence (EUROFORGEN-NoE), funded by the European Commission within the 7th Framework Programme (Grant Agreement No. 285487).

Application of multifactor dimensionality reduction analysis and Bayesian networks for eye color and ancestry prediction for forensic purposes in Czech Republic

A. Zidkova[1], A. Horinek[1], M Korabecna[1]

[1]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Advances both in genotyping technology and human genetics resulted in forensic molecular phenotyping. Here we would like to present model based approach to create panel of SNP markers for eye color and ancestry prediction.

Population sample contained 131 unrelated individuals: 100 (76%) Caucasians and 31 (24%) Asians (Kazakh). Samples were binned according to eye color: 47 (36%) light eye color and 84 (64%) dark eye color. Genotyping was performed by end point PCR method using TaqMan MGB assays. All samples were analyzed using panel of 22 SNP markers selected from literature. Data were divided into two sets: the training (100 Caucasians, 31 Asians) and the testing (47 Caucasians, 81 Asians). Data for testing set were obtained by following methods: Caucasian data by genotyping, Asian data were extracted from HapMap. Firstly, SNP data of the training set were filtered using ReliefF, which minimizes risk of SNP false positive association with studied phenotype. Consequently, multifactor dimensionality reduction was performed to uncover possible epistatic regulation between studied loci. For validation of selected markers Bayesian network was constructed.

Constructed network contained ancestry determination using SNP markers rs1426654 and rs16891982 and eye color determination using rs12913832, rs7495174 and rs916977. Sensitivity of the model reached 0.96, specificity was 0.99. Prediction of eye color using presented Bayesian network could be useful in case of unknown body remnants identification and verification of eye witness testimony.

Supported by the grant TIP No. I/328 of the Ministry of Industry and Trade of the Czech Republic.

Distribution of likelihood ratios in complex immigration cases

M. Hedman[1], J. U. Palo[1]

[1]Laboratory of Forensic Biology, Department of Forensic Medicine, University of Helsinki, Finland

In Finland, as well as in several other countries, residence permits can be granted on the basis of family ties. However, many applicants lack documented proof of their familial relationships; in these cases immigration services often rely on kinship analyses based on DNA profiles.

In immigration cases DNA tests are often requested in complex cases, such as to evaluate probabilities of sibship or half-sibships. Unlike for e.g. standard paternity cases, there are no generally accepted guidelines as how to interpret the obtained likelihood ratios in sibling or half-sib scenarios. Furthermore, relatively high number of aSTRs is needed for reliable results, but when the number of analyzed loci increases, the chance of finding a mutation increases as well. Here, we have analyzed 100 families (mother, father and at least two common children) to assess the distribution of likelihood ratios in true siblings and half-sibs. This also allowed evaluation of the impact of a mutation on sibship and half-sibship likelihood ratios.

Using 15 aSTR markers (AmpFlSTR Identifiler), widely overlapping

likelihood ratio distributions were obtained for fullsibs, half-sibs and even unrelated offspring, urging caution in interpreting the results. An order of magnitude lower LRs were obtained in true full sib-pairs with observed mutation, as contrasted to pairs with no mutations.

DNA tests are reliable method to prove biological relationships in immigration cases, but it is limited by the samples of the family members that are available. Also the nature of STR markers (mutations, population specific allele frequencies) challenges the interpretation of the obtained results.

Complex DNA-mixtures with an unspecified number of contributors

N. Kaur[1], T. Egeland[1,2], the EUROFORGEN-NoE Consortium

[1]Norwegian university of Life Sciences, Ås, Norway

[2]Norwegian institute of Public Health, Oslo, Norway

When the number of contributors in a DNA-mixture exceeds more than two persons, we need more robust methods to determine whether a specific individual has contributed to the mixture. Rape cases where several suspects are involved (e.g. gang rape) is an important example in the forensic casework where better methods are needed to interpret the evidence.

The conventional methods used in forensic casework today are often based on a limited number of markers gained from the crime scene. We show that power can be increased by rather using SNP-markers. New statistical methods are needed for this, and we present a regression model that more robustly handles SNP-markers.

Data from a controlled blinded experiment is used to derive a linear regression model for solving mixture cases. With this model, testing whether a specific individual has contributed to a DNA-mixture is reformulated in terms of regression coefficients. The model does not require the number of contributors to be specified, and the hypotheses are formulated in terms of the amount contributed by a suspect.

Simulations are performed to test the model, and the data from the experiments are analyzed. The data consist of 25 DNA-mixtures, made by a varying number of contributors that contribute in different proportions. The analyses show that useful estimates can be obtained from a relatively small number of SNP-markers (around 300). Power calculations indicate that individuals contributing as little as 1% to the mixture can reliably be detected when 500,000 SNP-markers are available.

Free open source software for internal validation of forensic STR typing kits

O. Hansson[1], P. Gill[1,2], and the EUROFORGEN-NoE Consortium

[1]Norwegian Institute of Public Health, Department of Forensic Biology, Norway

[2]University of Oslo, Norway

The validation of new short tandem repeat (STR) systems for forensic purposes is extremely time consuming and expensive. However, if a full understanding of biological processes was achieved, then this would effectively by-pass the need to carry out validation by traditional methods, since millions of DNA profiles could realistically be generated in-silico at no cost. To achieve this, a PCR simulation tool and a validation toolbox have been built using 'R programming language'.

The goal is to provide realistic outputs of virtual DNA profiles by simulation of the specific methods (extraction, PCR and electrophoresis) that are used routinely in the analytical method used.

With accurate simulations it will be possible to create virtual DNA profiles including real casework examples (such as partial mixtures and degraded samples). Ultimately the program will be used to assist experimental design e.g. to define the best parameters to analyse a sample. Also the output could potentially be used in probabilistic analysis.

The validation toolbox aids the implementation of new kits by simplifying the analysis of validation data. It provides functions to explore the characteristics of DNA typing kits according to ENFSI recommendations (e.g. balance and stutters). It facilitates the comparison of simulated and real data, and is therefore an important tool to 'fine tune' the parameters used for simulation. Both packages are open source and have easy-to-use graphical user interfaces. Command line functions are still available for power-users.

Simulated approach to estimate the numbers and combinations of known/unknown contributors in mixed DNA samples using 15 short tandem repeat loci

S. Manabe[1], C. Kawai[1], K. Tamaki[1]

[1] Department of Forensic Medicine, Kyoto University, Graduate School of Medicine, Kyoto, Japan

The calculation of likelihood ratios (LRs) for DNA mixture analysis is necessary to establish an appropriate hypothesis based on the estimated number of contributors and known contributor genotypes. Here, we recommend a relevant analytical method derived from the 15 short tandem repeat typing system (the Identifiler multiplex), which is used as a standard in Japanese forensic practice and incorporates a flowchart that facilitates hypothesis formulation. We postulated that: (1) all detected alleles need to be above the analytical threshold (e.g., 150 RFU); (2) alleles of all known contributors should be detected in the mixture profile; and (3) there should be no contribution from close-relatives. Furthermore, we deduced that mixtures of four or more persons should not be interpreted by Identifiler as the LR values of simulated 100,000 cases have lower expectations for exceeding our temporal LR threshold (10,000) which strongly supports the prosecution hypothesis. Thus, DNA mixtures composed of four or more persons cannot undergo further estimation using our approach. We validated the method using various computer-based simulations and found that the estimated number of contributors is most likely equal to the actual number if all alleles detected in the mixture could be assigned to those from the known contributors. By contrast, if an unknown contributor(s) needs to be designated, LRs should be calculated from both two-person and three-person contributions. We should also consider some cases in which the unknown contributor(s) is genetically related to the known contributor(s).

A user friendly likelihood ratio (LR) calculator that estimates and incorporates the probability of dropout (PrD).

T. Kalafut[1], J. Sutton[1], J. Buckleton[2], J.A. Bright[2], L. Armogida[3]

[1] United States Army Criminal Investigation Laboratory

[2] Institute of Environmental Science and Research Limited, Auckland, New Zealand

[3] NicheVision Forensics, LLC

The International Society of Forensic Genetics (ISFG) recently recommended the use of probabilistic methods for interpreting forensic DNA profiles, including incorporation of the probability of dropout (PrD). The incorporation of PrD calculations into a likelihood ratio (LR) framework is complicated by the nature of evaluating PrD. This paper describes the development of software that calculates

both the logistic regression curves that are needed to accurately estimate PrD and the final LR for two and three person mixtures. The regression curves are calculated directly by analyzing an appropriate number of low level known samples that exhibit dropout. These results are calculated using a validation module that is a part of the LR calculator. This calculator can be easily validated by any laboratory using a set of samples created with their own protocols. The calculator is seamlessly integrated into ArmedXpert, a user friendly forensic data management and analysis system.

How the automation of DNA data interpretation using ArmedXpert software has benefited the United States Army Criminal Investigation Laboratory (USACIL).

T. Kalafut[1], J. Sutton[1], L. Armogida[2]

[1] United States Army Criminal Investigation Laboratory

[2] NicheVision Forensics, LLC

The USACIL conducts forensic DNA examinations for Department of Defense investigations worldwide. As such, over 70 DNA examiners and contractors are normally staffed at one time both stateside and in theatre. These individuals all have different backgrounds and experience levels yet work under similar protocols. Software developed in house has been invaluable in streamlining the analysis and interpretation process. It has also provided a relatively high level of consistency in final results as determined by the statistical analysis of mixed DNA profiles used during internal competency testing. As this software was developed over time in our laboratory based on the input of many examiners, we feel it is a complete tool for all aspects needed to go from allele calls to final report. In addition to tremendous time savings, it has eliminated things such as transcription errors, arithmetic errors, highlighters, and pages of manual calculations that traditionally hamper the interpretation process. Features include control checks, contamination checks (over time and across co-extracted cases), and comparison of non-matching profiles to staff data bases or anomalous results profiles. It can also provide documentation of all matches/inclusions, find additional foreign alleles, and provide peak height ratio and mixture proportion information for the deconvolution of two and three person mixtures. Statistical options include CPI, RMP (including samples with stochastic issues), LR and LR incorporating Pr(D). In a real-time demonstration, presenters intend to complete the analysis of a recent proficiency test from .fsa files to the submission of final results in a matter of minutes.

Separation of mixed biological forensic samples using FACS

T. Verdon[1,2], R.J. Mitchell[1], R.A.H. van Oorschot[2]

[1] Department of Genetics, La Trobe Institute for Molecular Sciences, Melbourne, VIC, Australia

[2] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Division, Macleod, VIC, Australia

Mixed biological samples can pose significant problems when examined with traditional forensic analytical techniques. Fluorescence assisted cell sorting (FACS) was proposed as an alternative to differential lysis for separating sperm from epithelial cells in 1999. Advances in FACS technology since have provided increasingly sophisticated methods which can possibly be applied to mixtures of other cell types of forensic interest. In this study we demonstrate the potential for separation of mixtures using flow cytometry.

Fresh blood and saliva were mixed in ratios of 1:1000, 1:100, 1:50, 1:10, 1:5 and 1:1 for both blood:saliva and saliva:blood. 100 µL aliquots of mixtures were tagged with fluorescently-labelled antibodies specific to surface antigens of white blood cells (CD45) and mucosal epithelial cells (CD227). Cells were subsequently sorted based on size and fluorescence, using FACS selecting for large $CD227^{+}/CD45^{-}$ buccal epithelial cells or smaller $CD45^{+}/CD227^{-}$ leukocytes. DNA was extracted from each sample, quantified and genotyped.

The number of alleles derived from the minor donor was, in most cases, substantially increased by FACS compared to an unsorted sample of the mixture. Although not always removing all alleles of the major donor, the percentage of the resulting mixed profile attributed to the minor donor was increased, as were likelihood ratios. Although further work is required to translate this application to analysis of dried samples, and also for use with cells other than leukocytes and mucosal epithelial cells, this preliminary study demonstrates enhanced potential of FACS since the initial proposed forensic application in 1999.

MIXplex: can a gonosomal marker multiplex aid in mixture analysis?

T.M. Diegoli[1,2,3], A. Linacre[3], and M.D. Coble[4]

[1] Armed Forces DNA Identification Laboratory, Armed Forces Medical Examiner System, Dover Air Force Base, DE, USA

[2] American Registry of Pathology, Camden, DE, USA

[3] Flinders University, Adelaide, SA, Australia

[4] National Institute of Standards and Technology, Gaithersburg, MD, USA

Mixture interpretation is an important part of the forensic scientist's role in evaluating evidence from a crime scene, where mixed stains can be common. To this end, a mixture multiplex has been created that combines markers from both the X and the Y chromosomes in an attempt to aid in the interpretation of such mixtures, providing clues as to the number of contributors and the sex of those contributors. By maximizing the information gained from these mixtures, the direction of further testing could potentially be influenced and optimized.

Preliminary testing of the mixture multiplex, or "MIXplex," showed consistent sensitivity down to at least an 80:20 mixture or 200 pg input. All amplicons were designed to be less than 200 bp in order to increase the potential for success with challenging samples. Artificial mixtures composed of different combinations of males and females, varying ratios of contributors, and increasing numbers of contributors were created and typed with both the MIXplex and a commercially available autosomal STR kit. Results were compared to determine the benefits and limitations of using gonosomal STRs in the evaluation of mixed evidence in the forensic setting.

This work was supported by U.S. National Institute of Justice grant award 2011-DN-BX-K401.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense, the United States Department of the Army, or the National Institute of Justice.

The effect of using wildcards in forensic DNA database searches

T. Tvedebrink[1,2]

[1] Department of Mathematical Sciences, Aalborg University, Aalborg, Denmark

[2] Section of Forensic Sciences, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction Forensic genotyping is used as evidence and lead generating tool in many instances of the judiciary system. Over the recent years, the national databases of DNA profiles have grown in size due to the success

of forensic DNA analysis in solving crimes.

Aim The accumulation of DNA profiles implies that the probability of a random match or near match of two randomly selected DNA profiles in the database increases. These reported near matches between supposedly unrelated individuals has caused some concern in the general public. However, Weir (2004, 2007) demonstrated elegantly that these near matches are close to what one would expect based on very simple population genetic models.

Materials and methods Tvedebrink et al. (2012) derived computational efficient expressions for calculating the expectation and covariance of the near matches statistic for a given DNA database. In this work we show how the use of wildcards affect these quantities, and implement this in a R-package (DNAtools) for analysing DNA databases.

Results and discussion If one of the two different alleles at a heterozygous locus fail to be typed, a truly heterozygous profile will be typed as homozygote, which may imply this individual to be excluded from further investigations. In order to avoid such exclusions of DNA profiles due to mistyped heterozygous loci, the use of wildcards has been proposed and used by several national agencies. We show how the use of wildcards affect the probability of matches and near matches in a forensic DNA database.

Exact calculation of the distribution of the number of alleles in DNA mixtures

T. Tvedebrink[1,2]

[1] *Department of Mathematical Sciences, Aalborg University, Aalborg, Denmark*

[2] *Section of Forensic Sciences, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

DNA mixtures are frequently occurring in forensic case work. The number of contributors to a given stain is in principle unknown. The number of observed alleles together with their signal intensities is used to assess the number of contributors. However, the distribution of the number of alleles for a given number of contributors and STR loci has not been derived in exact form.

Ongoing work in forensic genetic software utilise the number of observed alleles in order to estimate the probability of allelic drop-out together with a plausible range. The current methodology relies on simulations of the number of alleles for a given number of profiles. We present a method for computing the distribution of the number of alleles for any number of contributors and number of loci.

By mathematical recursion equations, we are able to express the computation of the number of alleles in an efficient programmable set-up. Corrections for sub-populations effects are also implemented in this recursive computation scheme. An implementation in the statistical software R demonstrate that the method is computational fast and accurate.

We have evaluated the methodology on Danish STR allele frequencies for up to seven-person DNA mixtures. Consequently, when the number of observed alleles is used to assess the probability of allelic drop-out, this quantity is fixed for any DNA stain with identical number of observed alleles – irrespective of the signal intensities. Hence, this non-intuitive construction implies that the drop-out probability can be tabulated for fixed number of contributors and loci.

Theme 12: Forensic DNA Typing

Recovery of partner's DNA after intense kissing

F. Alessandrini[1], M. Pesaresi[1], A. Tagliabracci[1]

[1] *Dipartimento di Scienze Biomediche e Sanità Pubblica, Sezione di Medicina Legale, Università Politecnica delle Marche, Ancona, Italy*

Identification of foreign biological material by genetic profiling is widely required in forensic DNA testing in different cases of sexual violence, sexual abuse or sexual harassment. In all these kinds of sexual assaults, the perpetrator could constrain the victim to kissing. The value of the specimens collected from victim's mouth (perilabial and labial sites, theeth and tongue) taken after the crime has not been investigated with currently used molecular high sensitivive and specific methods.

Ten voluntary pairs were tested at various intervals after intense kissing and samples were taken frome the above mentioned sites to assess the presence of partner's DNA. Extracted DNA was quantified using the Promega Plexor HY assay and autosomal and Y-STRs were analized.

Our study confirms that foreign DNA tends to persist expecially in the perilabial and labial sites, while rapidly disappear in the mouth (theet and tongue). If prompt collected, the biological material can be used as a valuable source of evidence. In cases where samples are collected from a living victim as evidence material, the crucial factor that can influence the success of analysis is the time between the criminal act and sample collection.

Detection of DNA within Fingermarks

A. Haines[1], S.S. Tobe[1], H. Kobus[2], A. Linacre[1]

[1] School of Biological Sciences, Flinders University, Adelaide, Australia

[2] School of Chemical and Physical Sciences, Flinders University, Adelaide, Australia

DNA is deposited onto a surface by touch yet few means have been developed for its in situ detection. A range of dyes are available that bind to DNA at high specificity and here we report on the use of one of these dyes to detect latent DNA. SYBR® Green I was used to detect DNA within fingermarks. Fingers and thumbs were pressed onto a range of substrates such as plastic and glass to create a fingermark. A range of donors were chosen to account for variability in the deposition of DNA. A solution of dye was then pipetted onto the mark and allowed to dry briefly. Emission of fluorescence at 520 nm was detected at the position of the mark. No fluorescence was observed around the fingermark or in the negative controls, indicating the dye bound to DNA present within the fingermark. Variability was noted based on individuals and activity prior to creating the mark. By altering the volume and concentration of dye, ridge detail within the fingermark could be observed allowing the possibility of not only detecting latent DNA but also using this method for human identification and fingermark comparison.

Funding was provided by the Ministry of Justice, South Australia

Bitemarks in foodstuffs - an approach for genetic identification of the bitter

A.Corte-Real[1], Vanessa Bogas[2], Duarte Rodrigues[1], F. Corte-Real[1,2], M. João Porto[2]

[1]Faculty of Medicine, University of Coimbra

[2]Central Branch, National Institute of Legal Medicine and Forensic Sciences

Biological evidences from partially eaten food, left in crime scene, may contain genetic information of the bitter that can generate a DNA profile. The aim of this paper is to study two different methods to obtain the DNA on foodstuff.

15 apples were cleaned with sodium hypochlorite before to be bitten by the same individual. Then, they were stored and allowed to dry at environment temperature for 24 hours. Each bitten apple had been previously divided vertically in two equal size parts. In one of the two areas, DNA was collected from the inner and in the other part from the periphery. For both of them double swab technique was performed. DNA extraction was executed by the using PrepFiler™BTA kit and quantification was made with Quantifiler™DUO kit, both from Applied Biosystems, according to the manufacturer's instructions. The results were associated with the study viability of the polymorphisms for identification (European standard set).

Quantification values stayed between 0,00219 ng/µl to 1,7054 ng/µl. Difference among the two methods was proved. In human skin we perform this technique in the middle of a bite mark, as well as in previous studies in which the DNA was collected from the inner of foodstuff with lower quantification. This new experimental condition of collected DNA at periphery allowed to conclude that through collection of epithelial lip cells escaped from apple enzymatic activity.

Key words: bitemark, genetic profile, identification

Post Mortem sampling of the bladder for the identification of victims of fire related deaths

A. Schlenker[1] , R. Owen[1], P. Bedford[1], J. Leditschke[1, 2], and D. Hartman[1,2]

[1] Victorian Institute of Forensic Medicine, 57-83 Kavanagh St, Southbank VIC 3006, Australia.

[2] Department of Forensic Medicine, Monash University, Australia.

The Victorian Institute of Forensic Medicine (VIFM) is a purpose built facility providing forensic medical services to the State of Victoria, including scientific evidence for the identification of deceased persons who cannot be visually identified. Using DNA analysis, the post-mortem (PM) identification of deceased persons relies on the retrieval of a suitable PM sample.

Depending on the condition of the body, the PM sample collected for analysis will vary – usually blood, muscle or bone are taken. Some cases, however, can prove difficult; these include cases of severe decomposition or incineration, which may require multiple sample types be collected for analysis. The identification of incinerated bodies can be particularly challenging, as bones may become brittle or be severely affected in their ability to yield DNA for analysis.

The VIFM has evaluated the use of bladder swabs for coronial investigation involving victims of fire [1]. The bladder swabs proved to be an effective source of DNA from victims of fire when compared to the conventional sample collected. Furthermore, the collection of bladder swabs was less invasive and less time consuming. The bladder swabs also require less processing compared to other sample types such as bone (which require sample pre-treatment before DNA analysis can commence). This presentation will discuss our findings for the use of bladder swabs as a primary identification sample at the VIFM, with the authors recommending the collection of bladder swabs for cases involving fire.

"Investigation of the use of bladder swabs as samples for the DNA identification of incinerated deceased persons" VIFM Ethics Approval No: EC 3/2012.

The potential transfer of trace DNA via high risk vectors during exhibit examination

B. Szkuta[1,2], M.L. Harvey[1], K.N. Ballantyne[2], R.A.H. van Oorschot[2]

[1]Deakin University, Geelong, Victoria, Australia

[2]Victoria Police Forensic Services Department, Macleod, Victoria, Australia

As the sensitivity and discriminative power of DNA technology elevates, the use of trace DNA as evidence in forensic casework also rises. Along with increased sensitivity comes the prospect of detecting contaminating DNA, complicating the interpretation of profiles and possibly inculpating innocent individuals in criminal activities.

This investigation considers the occurrence and level of DNA potentially transferred between high-risk vectors (scissors, forceps, gloves) and exhibits during the examination process. Mock forensic casework exhibits comprised of cotton swatches containing a dried bloodstain (primary substrate) and others which were DNA-free (secondary substrate), each measuring 1.5 × 1.5 cm. Contact occurred on both sides of the substrate and consisted of scissor cuts spanning the length; medium pressure touches with a 1cm forceps tip; or medium pressure touches by gloved thumb and index fingers. Each touch lasted 3 sec. Primary substrates were cut or touched eight times or once, followed by the same action to the secondary substrate. DNA was extracted from the secondary substrate, quantitated, and then profiled using PowerPlex® 21 (Promega).

DNA transfer was observed for each vector in both multiple and singular contact situations; multiple contact scenarios transferred more than singular. Profiles with sufficient alleles to identify the origin were observed within both situations for each vector except forceps, where only a few alleles were transferred in singular contact situations.

The findings of this study provides an indication of potential contamination levels when laboratory equipment is not cleaned or replaced as required, and an assessment of DNA contamination risks during examination.

The Recovery of Offender DNA from Victim Skin- Evaluation of Sampling Techniques for PP21 Analysis

B.Chapman[1], M. Reynolds[1], J Bennett[2]

[1]Forensic Field Operations- Crime Scene Unit. Western Australia Police, Midland, Western Australia

[2]PathWest Laboratory Medicine WA. QE2 Medical Centre, Nedlands, Western Australia

Often in the commission of serious assaults and murder, an offender will have vigorous contact with the victim, either during the physical assault or post-mortem in order to conceal the crime. Very little has been published to suggest what may be the most effective method of recovering this trace evidence, particularly DNA deposited onto common contact areas such as wrists, ankles and necks. In this study we have compared the traditional method of swabbing to a number of tape lifting based methods. Previously the concern of overloading offender DNA with that of the victims has been a concern but with newer profiling kits the ability to resolve mixtures becomes easier.

A number of mock scenarios were designed and three adhesive tape products were compared to the existing foam swab methodology to

ascertain best offender DNA yields. Additionally a case study from an actual homicide scene was used as a trial proof of concept.

Results are pending at the time of abstract publication.

Comparison of three methods for extraction of DNA from formalin fixed paraffin-embedded tissues

L. Chao[1], L. Jin[2], C. Xiao-hui[1], L. Chang-hui[1]

[1] Guangzhou Forensic Science Institute, Guangdong Province Key Lab of Forensic Genetic

[2] Institute of Forensic Medicine, Southern Medical University, Guangzhou

Formalin-fixed and paraffin-embedded tissue is an important kind of personal files in the department of pathology and forensic institutions. It is also an important biological material source in oncology research. The tissue treated by formalin and paraffin can easily cause DNA cross-linking and degradation which can bring great difficulties to the extraction of DNA, and even interrupt the research. How to extract high quality DNA from formalin-fixed and paraffin-embedded tissue has been the great problem plagued researchers. The purpose of this paper is to explore an optimal method for extraction of DNA from formalin fixed paraffin-embedded tissues.

We collected 20 formalin-fixed and paraffin-embedded cancerous tissue specimens, each specimen was cutted into 9 slices of 3 micron thick, and divided into three groups according to the method for dewaxing. The specimens in each group were treated by Chelex-100 combined with QIAquick®PCR Purification Kit, DNA IQ™ kit, Chelex-100 combined with DNA IQTM kit, respectively. The quality of obtained DNA was analyzed by PCR quantification.

The yield of DNA dewaxed by xylene and extracted by Chelex-100 combined with purified by QIAquick®PCR Purification Kit is significantly higher than those treated by other methods.

Xylene is very suitable for deparaffinage, and Chelex-100 extraction combined with QIAquick®PCR Kit purification is very suitable for extracting DNA from formalin fixed paraffin-embedded tissues.

Human bones from 1772 to 1850 uncovered in archaeological excavation in Porto Alegre, Brazil: DNA identification, morphological and historical studies.

P.E. Raimann[1,2], J.B. Picanço[1], G. Hirschfeld-Campolongo[2], F.B. Souza[1], P.C. Concaves[1], F.B. Tocchetto[3], A.M. Cappelletti[4], C.S. Alho[1]

[1]Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

[2]Life Technologies, São Paulo, SP, Brazil

[3]Museu Joaquim Furtado, Porto Alegre, RS, Brazil

[4]Cúria Metropolitana, Porto Alegre, RS, Brazil

The history of Porto Alegre officially began in 1752, when approximately 500 Azorean citizens were given land and occupied its territory. The city was funded in 1772 with approximately 1,500 inhabitants. In this period the local government house, a cemetery, a prison, a theater, and a catholic cathedral were built. In 1798 there were 3,000 inhabitants and in 1814, 6,000. In September 2011 the original cemetery with the first inhabitants of Porto Alegre was discovered. The archaeological excavation revealed above 90 human skeletons dating from 1772 to 1850 buried directly in the ground. Materials and methods: Molecular, morphological, and historical analyses were conducted on the remains. Results and discussion. The DNA analysis showed profiles in STR autosome loci. The morphologic study determined that there are both male and female remains of different age classes and two children. The historical survey suggests that they belonged to notable politic-social-religious persons with Iberian origin, as well as native indians or African slaves. In the sepultures were found shroud spangles, necklace beads (typical of slaves), bony tooth brushes, pipes, a military bottom of the Dom Pedro II (Brazilian Imperor) army, fragments of indigenous ceramics, etc. Access to the DNA was of critical importance due to the challenge of ancient and ill-preserved samples with high historical value. The exploration of autosomes, mtDNA, and Y-STRs data will be capable to reveal who were those people. This is the first time that DNA analyses help to uncover the past history of an urban community in South Brazil.

Qualifying genetic profiles in challenging samples: bones as a test case

M. Caputo[1,2], D. Corach[1,2].

[1]Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina.

[2]CONICET (National Scientific and Technical Research Council), Argentina.

Success in DNA extraction methods is often determined by quantifying DNA yields and determining the number of loci that provide reliable genotypes. Aiming to develop objective criteria to evaluate DNA profile quality, allele/locus drop in/out, heterozygote imbalance and increased stutter percentage were analyzed in genetic profiles obtained from challenging samples.

PowerPlex16HS® profile quality from bone samples submitted to two preparing approaches: A- fine powder (n=29) and B- thin slices of bone (n=32), were evaluated using GeneMapper ID-X version 1.0 and analyzing numerical indexes such as: total peak height (TPH), intralocus balance (MLB) and inter-loci balance estimated by Shannon Entropy (SH).

Development of new methodologies requires adequate qualifying criteria for objective comparisons. DNA quality profiles from two bone preparations (A and B) were compared. In average, 13 loci were successfully determined for method-A and 9 for method-B. Additionally, for method A, a drop-in of 16.6%, 3% of drop-out, 8.2% of increased stutter percentage and 10.8% of not determined locus were obtained in contrast to method B: drop-in 1.6%, drop-out 3.8%, increased stutter percentage 1.4% and not determined locus 9.3%. Regarding numerical indexes, in profiles obtained in A, the average TPH was 13025; MLB 0,81 and SH 1,73 contrasting to those obtained in B with average TPH of 20362; MLB 0,82 and SH 2,32. The quality measures demonstrated that better quality profiles were obtained when DNA was extracted from bone slices than from bone powder. These objective criteria might be used for comparing other genotyping steps whose final result are genetic profiles.

Evaluation of DNA extracting methods from formalin-fixed and paraffin embedded tissues

M. Caputo [1,2], M. Irisarri[1], A. Corach [1], D. Corach. [1,2]

[1]Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina.

[2]CONICET (National Scientific and Technical Research Council).

Human biopsies provide a valuable diagnostic tool. Processing by fixation, embedding in paraffin wax allows long term storage. Formalin-fixed and paraffin embedded (FFPE) tissues are a source for histological

examination, molecular diagnostic and forensic investigations; however, available material is often limited in quality and quantity. Here, we presented a semi-automated protocol for obtaining nucleic acids suitable for forensic analysis.

Twelve FFPE tissues were processed by a modified Maxwell Protocol (A), compared with classical Xylol/Proteinase digestion/Phenol-Chloroform method (B) and with a commercial kit ZR FFPE DNA MiniPrep™ (C). DNA was amplified by PowerPlex16^{HS}. The analysis was performed by Genemapper IDX software 1.0 and intralocus balance (MLB) and inter-loci balance estimated by Shannon Entropy (SH).

DNA quality profiles from three different extracting FFPE methods were compared. In average, 8 loci were successfully determined for method A, 6 for method B and 4 for method-C. Although the drop-out and not determined locus proportion were similar in the three methods, an increased of drop-in were observed in methods B and C. Regarding intra/extra locus imbalance, the results obtained for method A and B (0.76/1.3) were similar and better than C (0.63/0.67). Thus, the FFPE tissue extraction procedure proposed represents a semi-automated rapid alternative for obtaining DNA suitable for forensic identification. Additionally, RNA extraction optimization from FFPE tissues will add new tools in forensic research and is, at present, in progress.

Comparison of traditional cotton swabs to Copan 4N6FL0QSwabs™ for crime scenes evidences collection

M. Nelson[1], G. Caves[1], R. Santiago[1], S. Castriciano[2], and D. Podini[1]

[1] The George Washington University, Washington DC, USA

[2] Copan Italia, Brescia, Italy

Cotton swabs are often used to collect DNA evidence at crime scenes and in forensic laboratories. While highly absorbent, the dense inner core can trap cellular materials. An alternative type of swab, called 4N6FL0QSwabsTM (4N6FS) (Copan Italia, Brescia, Italy), is made of parallel short nylon strands that are flocked onto a plastic stick lacking an inner core that can trap cellular materials. The swab is also designed to neutralize microbial contaminants while preserving nucleic acids integrity without the need to dry the swab.

In this study known amounts of lymphocytes and buccal epithelial cell suspensions were spotted on various substrates in multiple replicas simulating forensic evidence (knife handle, gun grips, etc.). Samples were then collected with cotton and flocked swabs in parallel and extracted the same day and after 2-week storage to test the antimicrobial capability. Extraction was performed with the PrepFiler® Forensic DNA Extraction Kit (Life Technologies) and DNA IQTM System (Promega) with and without the use of a Nucleic Acid Optimizer (NAO), a semi-permeable basket, which retains fluid until placed in a centrifuge. Extraction yield was determined with Quantifiler® Human DNA Quantification Kit (Life Technologies) on an ABI PRISM® 7000 Sequence Detection System.

Best DNA recovery occurred when collection was performed with the 4N6FS swabs and extracted using the NAO in combination with the PrepFiler® kit. Furthermore the antimicrobial activity of the 4N6FS swab was confirmed.

Sub-sampling of human teeth for nuclear DNA analysis.

D. Higgins[1, 2], J. Kaidonis[2], G. Townsend[2], T. Hughes[2], J.J. Austin[1].

[1] Australian Centre for Ancient DNA, School of Earth and Environmental Sciences and Environment Institute, University of Adelaide, South Australia, 5005, AUSTRALIA.

[2] School of Dentistry, University of Adelaide, South Australia, 5005, AUSTRALIA.

Teeth are often instrumental to the process of human identification across multiple disciplines including anthropology, odontology and biology. In highly decomposed or skeletonised bodies teeth may be the most appropriate tissue for DNA analysis. However, the process of extracting DNA from teeth is a destructive process so must balance often competing requirements - maximising DNA profiling success whilst minimising impacts on features valuable to other disciplines and respecting the wishes of the next of kin. We aimed to determine the best source of nuclear DNA in teeth at varying stages of decomposition, focusing on targeted sampling of discrete tooth tissues. We quantified the yield of nuclear DNA from individual tissues using qPCR and explored the value of each tissue for genetic analysis from teeth that had been buried for up to 18 months. We demonstrate targeted sub-sampling of teeth is more conservative, preserves much of the tooth morphology and can maximize the success of genetic analysis. Sub-sampling of less mineralised tissues simplifies the laboratory processes and allows maintenance of the crown of the tooth, which can be replaced in the mouth.

Out of the Ordinary – Success Stories for Bones and Teeth

O. Handt[1], E. Formosa[1], D. Higgins[2]

[1] Forensic Science SA, 21 Divett Place, Adelaide SA 5000, Australia

[1] Forensic Science SA, 21 Divett Place, Adelaide SA 5000, Australia

[3] Australian Centre for Ancient DNA (ACAD), School of Earth and Environmental Science, The University of Adelaide, SA 5005, Australia

Bone and teeth are often the only available biological material for DNA identification of missing or unknown persons. Methods, specific to tissue type, designed to avoid lengthy cleaning processes and to allow rapid processing of samples were used to sample bones and teeth from a number of individuals. Cases investigated included an embalmed body and skeletonised remains believed to be around 30 years old. Best sampling practices and results obtained will be discussed.

Improved Recovery of Interpretable Results from Challenging Samples Using the GlobalFiler™ Kit

D. Wang[1], J. Mulero[1], M. Ludeman[1], L. Calandro[1], J. Schroeder[1], J. Tabak[1]

[1] Life Technologies, Foster City, CA, USA

Recent initiatives to expand international STR locus recommendations, most recently captured in the CODIS Core Loci Working Group's recommendation to expand the U.S. CODIS Core Loci from 13 to 20 "required" loci and 3 "highly recommended" loci, have crystallized the need for a highly discriminating global STR multiplex system. However, the design of such a system, which must include all the necessary loci without sacrificing performance and processing capabilities, poses significant challenges.

The GlobalFiler™ Kit was designed to meet these challenges and facilitate improved interpretation of challenging sample profiles routinely encountered in forensic casework, including inhibited, degraded, and mixture samples. This presentation will describe key design elements utilized to achieve this goal, including use of an additional fluorescent dye to maximize the number of smaller STR products and miniSTRs less than 220 bp in size, as well as formulation enhancements to enable increased sensitivity and inhibitor tolerance, clean baselines and high color balances while minimizing stutter peak heights, PCR or dye artifacts, and cross-reactivity with non-human species for ease of complex profile interpretation. Furthermore, three gender markers are included in a single dye channel, thus facilitating

male:female ratio determination and mixture interpretation.

Results from studies conducted internally and by multiple external test site laboratories will be shared to demonstrate increased recovery of alleles from a variety of challenging evidence sample types compared to previously utilized methods.

Further Development of DIP-STR Markers for Forensic Purposes

D Hall[1], J Gervaix[1], V Castella[1]

[1] Forensic Genetics Unit, University Center of Legal Medicine Lausanne and Geneva, Switzerland

DIP-STR are compound genetic markers that enable the analysis of biological stains containing highly unbalanced DNA mixtures from two individuals (ratios up to 1:1000). This novel analytical approach relies on pairing deletion/insertion polymorphisms (DIP) with STR. In this way, allele-specific primers overlapping the DIP sequence can target, under certain conditions, the amplification of the minor DNA contributor; while, the combined analysis of two polymorphisms generates a large number of alleles (DIP-STR haplotypes) suitable for identity testing.

Here, we discuss the latest results on DIP-STR marker developments to meet forensic standards including smaller amplicon size and longer tandem repeats, in addition to a Swiss population survey. Finally, we report results of both casework and simulated mixed samples resolution.

Besides providing a tool that improves the investigative value of a specific, and yet frequent type of samples; the lack of sex specificity and the simplicity of the techniques involved (PCR-CE) represent strong advantages over developing methods.

A novel TaqMan assay to accurately and efficiently detect the quantity and quality of human mtDNA in ancient, degraded or modern samples

E.Tobe[1], J.Austin [1], S.S. Tobe[2]

[1] Australian Centre for Ancient DNA, University of Adelaide, Adelaide, SA, Australia

[2] School of Biological Science, Flinders University, Adelaide, SA, Australia

Mitochondrial DNA (mtDNA) quantification has been problematic due to its minute size compared to the nuclear genome. Previous assays are based on total DNA values or estimate the quantity of mtDNA relative to nuclear and do not isolate the mtDNA prior to quantification. In addition, the quality of the mtDNA can affect what downstream assays are attempted.

We describe the development of a mtDNA standard and a novel TaqMan based assay to assess both quantity and quality of mtDNA. Three primer sets to amplify 85, 190 and 455 bp fragments were designed using 1,500 human mitochondrial genomes in addition to a synthetic ITC of 100 bp. Four fluorogenic probes were combined in a multiplex TaqMan assay. The ITC indicates any inhibition and the different fragment sizes provide an indication of the quality, as well as quantity, of any human mtDNA present. Samples with high quality DNA will show amplification for all three products, whereas degraded samples will not show amplification of the larger fragments, depending on the state of degradation. The mtDNA quantification standard was developed by purifying mitochondrial specific product under ultra-sterile conditions before quantification.

The assay was optimised using a modern human mtDNA sample before being tested on ancient and degraded samples that had previously been shown to contain highly degraded mtDNA. Modern mtDNA was also mixed with degraded samples to simulate results under 'contamination' scenarios. The developed assay will allow for accurate assessment of quantity and quality of mtDNA allowing for rapid and efficient analysis of samples.

Objective assessment of ninhydrin color reaction intensity required for DNA typing of epithelial cells on paper surfaces

E. Ochiai[1,2], K. Fukui[2], K. Maebashi[2], K. Sakai[2], M. Aoyagi[2], F. Satoh[1], M. Osawa[1], K. Iwadate[2]

[1] Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Japan

[2] Department of Forensic Medicine, The Jikei University School of Medicine, Tokyo, Japan

DNA profiling of desquamated epithelial cells adhering to a paper sheet is performed by staining the paper with ninhydrin and analyzing the extracted DNA from the ninhydrin-stained areas. The type of genetic marker examined, and the number of times the paper can be successfully analyzed, are subjectively determined based on the intensity and area of the color development. Here, we studied the relationship between the intensity of color development in the ninhydrin reaction and the limit of successful DNA profiling to objectively determine the target area on a sheet of paper. Eleven subjects rubbed their hands on white copy paper. The papers were then stained by ninhydrin, and the results were stored as digital images. The distribution of color density within a fixed area was measured, and used to calculate the density-intensity value (DI) by ImageJ image processing and analysis software. Regions with various DI values in a fixed area were cut out, and the DNA was extracted. The mitochondrial DNA (mtDNA) hypervariable region 1 was amplified by polymerase chain reaction and the mtDNA sequence was determined. Adhering cells in various DI areas were counted at six subjects. MtDNA extracted from white copy paper samples was quantified by real-time PCR. For white copy paper samples, the lower limit of DI at which the mtDNA sequence could be determined accurately in 50 % of cases was approximately 40,000 in 2.5 cm^2 areas. The success rate of DNA profiling increased as the intensity of the color produced by the ninhydrin reaction increased.

Noninvasive prenatal fetal genotyping from maternal plasma

E.M. Dauber[1], W.R. Mayr[1]

[1] Department for Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Austria

Cell-free fetal DNA circulating in maternal plasma is a target of prenatal RHD-genotyping by real-time PCR to determine the risk of D-alloimmunization of D negative mothers. Fetal cell-free DNA derives from apoptosis of the syncytiotrophoblast, which is of fetal genetic origin. The majority of cell-free DNA is of maternal origin deriving from the normal cellular turn-over in all maternal tissues. Therefore fetal DNA must be specifically amplified against a high background of maternal genetic origin. Prenatal genotyping of polymorphic markers used for human identity testing could presumably use the same source of fetal DNA. Therefore, different allele and haplotype specific amplification strategies were used for prenatal testing in two pregnancies (a female fetus in gestational week 25 and a male fetus in week 32). DNA was extracted from maternal plasma and eluted in a small volume. Typing of autosomal insertion/deletion (indel) polymorhisms and Y-chromosomal STR-typing was carried out by real-time and conventional PCR, respectively. In both cases fetal DNA was amplified successfully including a full fetal Y-STR haplotype. All non-maternal indel alleles found in maternal plasma were concordant with postnatal typing of the newborn. As a consequence, these noninvasive methods have the potential to be used for prenatal genotyping of polymorphic markers.

Chimerism in humans: challenge for forensic genetics

G. Chaudhary[1], A. Raina[1]

[1]DNA profiling Lab, Department of Forensic Medicine, Delhi, India

The role of forensic genetics within the investigative process is to compare sample recovered from crime scene with suspect. Forensic scientist may encounter the problem of mixed or completely mismatched DNA profiles of a single individual when the source of the biological material being analyzed is from a person who is a genetic chimera. Such genetic peculiarity may prevent the association of the perpetrator of an offence with the stain left at the crime scene or lead to false paternity exclusions. We analyzed three different biological specimens (Peripheral Blood, Buccal swab, Hair) at five different time interval (Pre-transplant, +21days, +3 months, +6 months, and +1 year) from twenty five different recipients of hematopoietic stem cell transplantation (HSCT), to estimate the chimeric status of patients. We used sixteen different STR markers to generate the genetic profile of the individuals. Donor chimerism was observed in blood (93-100%), buccal swab (2-57%) and hair follicle (0- 9.54%).

Identification of skeletal remains recovered from a seawall resolved a murder investigation case

G Calacal[1], J Salvador[1], SAE Franco[1], SM Maiquilla[1], M Sagum[1], MR Dalet[1], R Fortun[2], MC De Ungria[1]

[1]DNA Analysis Laboratory, Natural Sciences Research Institute, Miranda Hall, University of the Philippines, Diliman, Quezon City Philippines

[2]Department of Pathology, College of Medicine, University of the Philippines, Manila Philippines

Human remains identification is integral to criminal investigations. In the Philippines, there is reliance on testimonies and at times the only evidence presented in court. Hence, use of scientific evidence particularly DNA typing technology to aid in the identification of the sources of human samples during investigations is a most welcome development.

This report describes identification of a missing person whose skeletal remains were recovered from a seawall, eighteen months after her disappearance. Physical features and personal effects indicate consistency with the sex and build of the missing person with individualizing characteristics using dental records. A perforating gunshot wound of the head was the cause of death. The parents of the missing person believed that their daughter's father-in-law was responsible for her abduction and subsequent death. A case was then filed in court and DNA testing by a local laboratory was recommended.

A validated DNA extraction technique utilizing a detergent-washing step followed by an organic procedure was used to successfully recover DNA from the femur. The skeletal remains were exposed to adverse environmental conditions, e.g. intense tropical heat and humidity for an extended period of time. Autosomal Short Tandem Repeat (aSTR) profile at 15 genetic markers and mitochondrial (mtDNA) sequence at two hypervariable regions (HVI and HVII) were generated.

Conventional pathological and molecular DNA typing methods were used to identify that the recovered skeletal remains is that of the missing person, thus bringing closure to the victim's family. The case put forward the necessity for scientific evidence in Philippine courts.

Genome-Wide Methylation Profiling to Identify Body Fluid- and Age-Associated DNA Methylation Changes

J.H. An[1], A. Choi[1], K.J. Shin[1], W.I. Yang[1], H.Y. Lee[1]

[1]Department of Forensic Medicine, Yonsei University College of Medicine, Seoul, Korea

The ability to predict tissue type and donor's age from molecular profiles of crime scene samples has practical implications in forensics. A lot of studies have reported tissue-specific DNA methylation, but research to reveal the association between age and DNA methylation changes is being carried out to obtain more conclusive information. Here, we analyze DNA methylation patterns of body fluids (blood, saliva, semen, menstrual blood, and vaginal fluid) from 10 individuals aged 20 to 67 using the Illumina Infinium Human Methylation450 BeadChip array. We identified many CpG sites, whose methylation level is specific to a certain body fluid or strongly correlates with age. These results were confirmed by methylation SNaPshot or pyrosequencing analysis on 50 individuals or more. We found that DNA methylation patterns of semen were significantly different from the other body fluids and reveal much more age-related CpG sites than the others. In addition, most of the age-related markers were different between body fluids. Our results suggest that DNA methylation analysis at multiple CpG sites will allow simultaneous prediction of tissue type and donor's age of biological samples.

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (2012R1A1A2007031) and a grant from the National Forensic Service funded by the Ministry of Public Administration and Security.

Validation study of endogenous reference genes for normalisation of RT-qPCR data in post mortem skin tissue

I. Gomes[1], K. Becker[1], M. Rothschild[1], P.M. Schneider[1]

[1] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Cologne, Germany

Gene expression profiling may provide insights into the molecular mechanisms underlying wound skin repair contributing to applications in the field of post mortem forensic wound age estimation. Real-time quantitative PCR (RT-qPCR) is the most sensitive technique for gene expression studies but represents a complex procedure with several requirements for accurate data analysis. One of the major pre-requisites is the selection and validation of appropriate endogenous control genes for normalization in a given set of samples. In this study, the gene expression stabilities of the ten endogenous controls ACTß, ß2M, PPIA, GAPDH, HPRT1, PGK1, SDHA, TBP, UBC, and YWHAZ were evaluated in two sets of samples: post mortem human wounded and unwounded skin tissue aimed for subsequent wound age estimation studies. The most stable genes were determined by calculating a gene expression normalization factor for each sample based on the geometric mean of the ten selected genes using the reference gene validation software geNorm (Vandesompele *et al.*, 2002). In uninjured skin, the most stable endogenous controls were YWAZ, with the highest stability, followed by PGK1 and PPIA. In the wounded samples, GAPDH was the most stable gene followed by PGK1, ACTIN-B and HPRT1. Both groups revealed different reference gene expression stabilities with the exception of PGK1, which revealed to be the second most stable gene in both sample groups, and could thus serve as a candidate for a common reference gene. The different reference genes identified in both groups support the need for accurate normalization of gene-expression levels previous to RT-qPCR studies.

Where did that male DNA come from? A study of the transfer of male DNA in domestic situations.

C.F. Satur[1,2], H. Baker[1], J. Patel[1]

[1] Institute of Environmental Science and Research Limited (ESR), Auckland, New Zealand

[2] Forensic Science Program, Department of Chemistry, University of Auckland, New Zealand.

There is extensive research investigating the transfer and persistence of DNA using autosomal STR multiplex kits, but little data exists for using male specific Y STR profiling. In many sexual assaults the offender is known to the complainant and may either reside or have legitimate contact with them. To better understand the levels of male DNA detected using a Y STR DNA testing method, three everyday domestic activities (garment storage/laundering, skin swabs collected at the end of the day and wearing a garment) were investigated.

Here we present the results from 8 female participants. Using the Promega PowerPlex®Y (PPY) multiplex kit, the majority of the male results detected could be explained as originating from the male(s) the participant resided with. However, some foreign male DNA was also detected. Surprisingly no PPY results were detected in any of the skin samples with the exception of one participant who was known to have had physical contact with their partner. This demonstrates the need to exercise caution when interpreting Y STR results from garments. Conversely, detecting male DNA from social, domestic activities is more likely from there being a prolonged physical contact.

Genetic profiling from challenging samples

J. Templeton[1], R. Ottens[1], V. Paradiso[1], D. Abarno[2], O. Handt[2], D. Taylor[2], A. Linacre[1]

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

[2] Forensic Science SA, Adelaide, South Australia, Australia

The generation of a DNA profile from trace DNA has become the focus of much interest. Current methodology subjects the sample to a process to isolate the DNA, typically using a solid phase substrate. The aim of this study is to use direct PCR to generate both mitochondrial and STR profiles from latent DNA deposited by touch and also from samples considered to contain highly degraded DNA and unlikely to generate a profile if subjected to a DNA extraction process first. Direct PCR has the potential to: minimise loss of target DNA in a critical sample, omit steps involved in standard practice - which will significantly reduce labour time and cost, and increase the likelihood of obtaining a meaningful DNA profile for interpretation. Comparison between the results obtained by direct PCR and from extracts after standard extraction processes indicate the real potential use of the method described in this paper. The technique will have niche future applications in analysing degraded and low copy number DNA from samples that cannot be typed successfully using mainstream, STR-based, kits and protocols.

Funding for Jennifer Templeton, Renée Ottens, Viviana Paradiso and Adrian Linacre was provided by the Department of Justice South Australia.

Performance of the PowerPlexY23 kit on trace samples in forensic genetic casework

J.K. Olofsson[1], H.S. Mogensen[1], N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Y-chromosomal short tandem repeats (Y-STRs) can be analysed with the AmpF*l*STR®Yfiler® kit (Yfiler, Life technology (LT)) from trace samples in forensic case-work. However, the results of the Yfiler kit are not completely reliable when the ratio of female to male DNA is high, e.g. above 1,000:1. In addition, the balance of allele heights among the STR systems of the Yfiler kit is not completely satisfactory. The aim of this study was to investigate the performance of the

PowerPlex®Y23 kit (PPY23, Promega) on trace samples from crime scenes.

A total of 100-200 pg male DNA was amplified with the PPY23 kit from 45 trace samples. DNA amplification and fragment analysis was performed according to the recommendations of the manufacturer. When available, Y-STR profiles with the PPY23 kit were compared to Y-STR profiles previously obtained with the Yfiler kit.

Amplification of Y-STRs with the PPY23 kit was successful for 34 (76%) of the trace samples. No allele difference was observed for overlapping systems with the Yfiler kit. Systematic artefact peaks in the reading area (80-450 base pairs) were found in up to 29% of the trace samples typed with the PPY23 kit. Significantly higher female-to-male DNA ratios were observed in profiles with artefact peaks compared to those of profiles with no artefact peak. It was concluded that the quality of Y-STR analyses of trace samples would not be improved by exchanging the Yfiler kit with the PPY23 kit in our laboratory.

DNA analysis from gunshot residue (GSR) carbon adhesive stubs.

J. Castro[1], P. Zambrano[2], M. Salazar[1], M. Sosa[3], D. Sojo[3], Y. Ruiz[1], N. Guaimare[1], R. Gasperi[4]

[1]Genetic Forensic Laboratory, Ministerio Público, Venezuela

[2]Criminalistics Unit Against Human Rights Infringement Ministerio Público, Venezuela

[3]Electron Microscopy, Ministerio Público, Venezuela.

[4]Direction of Technical Scientific and Investigative Advisory, Ministerio Público, Venezuela

Gunshot residue (GSR) analysis by Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDX) it's the most powerful tool for forensic scientists to establishing to the identity of the shooter. GSR particles can be collected using adhesive stubs to study the morphology of the particles under an electron microscope. Aim: Evaluate obtaining DNA for genetic identification, from skin cells attached to an adhesive stubs used for GSR. Materials and methods: carbon-coated adhesive stubs where used to collect particles from the palmar and dorsal area from the hand after firing shots. Samples were analyzed with both scanning electron microscopy and energy dispersive X-rays. The carbon-adhesive was cut from the aluminum stubs with a scalpel. PrepFiler BTA Forensic DNA Extraction Kits was used to obtain the DNA from the epidermis cell on the adhesive, quantification by Quantifiler Duo on 7500 Real-Time PCR system all from Applied Biosystems. Blood from the same shooter on FTA Genecard (Whatman) was extracted using FTA extraction reagent, for comparison whit the GSR stubs. Autosomic profile was made with Identifiler Plus and allelic assignment on 3130xl Genetic Analyzer with GeneMapper software v3.2 (Applied Biosystem). Results and conclusions: it was possible to determine the complete genetic profile, from the palmar and dorsal area of the hand, was consistent with the genetic profile from blood sample, indicating that there is no effect from the GSR analysis with an electron beam on DNA typing.

Selective blood-DNA extraction from mixed stain using ABO antibody for short tandem repeat typing

K. Honda[1], T. Nishi[1], S. Yano[1], Y. Iwabuchi[1], Y. Sugano[1]

[1]Department of Legal Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba city, Japan

Analysis of DNA extracted from various kinds of mixed samples can be difficult to identify in criminal investigations. Here, we present a method for the extraction of DNA from mixed bloodstains involving plural contributors, followed by centrifugal separation of leukocyte agglutination by ABO antibody1.

We used a mixed blood sample originating from three men of different blood types (A, B, O) in a test tube. Another tube was kept and used for the mixed control (Tube N) without the selective extraction.

Anti-A antibody was added to Tube A, anti B-antibody was added to Tube B, and anti-H lectin strong was added to Tube O. After incubation of Tube A, B and H:O , the tubes were then centrifuged. The supernatant from the three tubes (A, B, O) was then aspirated and discarded including the extra antibody and non-cohered blood. Each pellet was then dissolved in TE buffer for DNA extraction.

DNA was extracted from A, B and O pellets by selective extraction and the mixed control (Tube N) using The Maxwell® 16 System. DNA typing were done using the PowerPlex® Y23 System.

We successfully recovered the DNA profile corresponding to the ABO blood type from the mixed sample. This method, together with the multiplex STR approach also presented, has proven highly successful in the recovery of DNA profiles corresponding to the ABO blood type.

DNA identification of skeletal remains by using a new extraction kit

K. Maeda[1], C. Murakami[1], M. Hara[2], W. Irie[1], M. Oishi[1], C. Sasaki[1], S. Nakamura[1], S. Takahashi[2], A. Takada[2], K. Saito[3]

[1]Department of Legal Medicine, Kitasato University School of Medicine, Sagamihara, Japan

[2]Department of forensic Medicine, Saitama Medical University, Iruma, Saitama, Japan

[3]Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan

The DNA extracted from tooth or bone would be progressively more fragmented and this result in a decreasing ability to gain a complete short tandem repeat (STR) profile. In this study, TBONE EX KIT (DNA Chip Research inc.) as a new extraction method, was applied to the samples from skeletal remains and extracted DNA were analyzed with AmpflSTR® MinifilerTM PCR Amplification Kit (Applied Biosystems) for DNA typing.

Tooth or bone was collected from 7 skeletal remains, which were 3 months to 15 years after death. The surface of the tooth or bone (0.5g) was removed and washed by ph- balanced detergent and ethanol in order to prevent contamination. These samples were frozen by liquid nitrogen and pulverized by Cryo- Press (Microtec Co., Ltd) before proceeding. DNA extraction was performed according to the manufacturer's protocol and purified using QIAamp DNA Mini Kit (QIAGEN). The genomic DNA was quantified using the QuantifilerTM Human DNA Quantification Kit (Applied Biosystems). STR typing was performed by AmpflSTR® MinifilerTM system in order to confirm genetic profile of the skeletal cases.

The DNA extracted by TBONE EX KIT showed concentration ranged between 0.023 to 25.71ng/µl from one tooth or 0.5g of bone. Moreover, all samples were completely genotyped at 8 loci using AmpflSTR® Minifiler™ system and the results confirmed the alleged relationship between missing person in 5 cases. Therefore, TBONE EX KIT would be a new applicable extraction method for forensic caseworks.

Linear Amplification of Target Prior to PCR for Improved Low Template DNA Results

K. Grisedale[1], A. van Daal[1]

[1]Bond University, Gold Coast, QLD, Australia

Traditional methods of low template DNA profiling often result in stochastic sampling effects exacerbated by the exponential nature of the PCR amplification. This study aimed to develop a pre-PCR procedure that increases the template copy number in a linear fashion which in turn could reduce stochastic effects.

Low template DNA samples with concentrations ranging from 100pg down to 6.25pg were divided into two aliquots and each half was subjected to a 10- or 20-cycle PCR, with the forward primer of an STR locus placed in one aliquot and the reverse primer of the same STR placed in the other aliquot. The single primer pre-PCRs then were pooled and used in a 30- or 35-cycle PCR with the primer pair.

Improved profiles were obtained using a 10- or 20-cycle pre-PCR step. The pre-PCR increased the overall recovery of alleles and resulted in higher average peak heights for all template amounts. The peak height ratios averages were similar for pre-PCR and control samples at 100pg and 50pg starting template amounts under the same PCR conditions. Greater variation in the peak height ratios was seen for lower template amounts. Results indicate that pre-PCR processing can increase the number of template copies available for the PCR amplification without adversely introducing stochastic sampling effects when at least 50pg of starting template are available. Improvements are also seen for lower template amounts, where allele and locus drop out still occur but at reduced levels in the pre-PCR samples compared to controls.

DNA Identification using FTA-Cards as Post Mortem Samples in a Plane Crash in the Northern Part of Sweden

K. Montelius[1], G.Pettersson[2], H. Nilsson[1], S. Sprogoe[2]

[1]National Board of Forensic Medicine, Dept. of Forensic Genetics and Forensic Toxicology, Linköping, Sweden

[2]National Board of Forensic Medicine, Dept. of Forensic Medicine, Umeå, Sweden

DNA-samples on FTA-cards are convenient, cost-effective and analyzed by many forensic labs. The possibility to extend the method to post mortem (PM-) samples would facilitate and speed up the DNA-identification process, compared to conventional methods. Here, we describe where PM-samples on FTA-cards were used for identification following a plane crash.

March 15 2012, a Hercules C130J, bound for Kiruna, Sweden, flew into the Kebnekaise area and descended to 2,000 m above sea level. The Kebnekaise summit rises to 2,104 m and the crash was inevitable. The Disaster Victim Identification (DVI)-team began the careful work of finding all remains from the crash, a work that continued through August. Human remains still embedded in ice and snow, were sent to the Department of Forensic Medicine in Umeå, where each finding was carefully thawed and examined. Duplicate samples, collected as tissue and as a swab on a FTA-card, were forwarded to the Department of Forensic Genetics for identification. Discs 1,2 mm in diameter were punched out of the FTA-cards with a BSD 600, amplified with Identifiler Direct and analyzed in an ABI 3500xl. In total, 160 FTA-card samples were analyzed. The success rate for the FTA-cards was 96 % for springtime samples and 80% for samples found in August, compared to 99% success rate for tissue samples. We conclude that PM-samples on FTA-cards are an efficient alternative to more conventional DNA-identification methods. However, a few degraded samples failed on FTA-cards, probably due to the limited amount of material on the card.

Sex determination problems in forensic genetic analysis

L. Laverde[1]

[1]. Genetic Laboratory of National Institute of Legal Medicine and Forensic Sciences, Medellin, Colombia, South America

Sex determination is important in forensic cases of missing persons, rape or to establish the sexual identity.

There are molecular tools to determine the sex such as the simultaneous quantification of autosomal DNA and Y chromosome and the tipification of amelogenin and Y chromosome markers but do not always yield accurate data on the determination of whether the individual is male or female and this can be related to mutational effects. Also in mixtures profiles cases, where there is a female predominant profile, may have problems in identifying the male component in the amelogenin and not always is successful the amplification of Y chromosome specific markers whose outcome can confirm the presence of a male profile.

In this research were analyzed different cases in our laboratory related to sex determination associated with mutations in the amelogenin sex marker or in theirs primers annealing regions, hermaphroditism, aneuploidies and the potential results of the forensic genetic routine markers whose interpretation should be used carefully to avoid misunderstanding in our reports and from the possible discrepancies between the biological gender and the (forensic relevant) legal gender in the personal identity documents that should not be underestimated.

Also is necessary to seek additional molecular tools to give reliable results related to sex determination.

Genetic identification of biological samples buried in clay soil during Summer and Winter seasons

V. Bogas [1], M. Carvalho [1,2], L. Souto [2], M.J. Anjos [1,2], M.F. Pinheiro [2,3,4,5], F. Corte-Real [2,6,7]

[1]Forensic Genetic Service, Centre Branch, National Institute of Legal Medicine, IP, Coimbra – Portugal

[2]CENCIFOR, Forensic Sciences Centre, Portugal

[3]Forensic Genetic Service, North Branch, National Institute of Legal Medicine, IP, Porto - Portugal

[4]Faculty of Health Sciences of University Fernando Pessoa, Porto – Portugal

[5]Faculty of Biomedical Sciences "Abel Salazar", Porto - Portugal

[6]Centre Branch of National Institute of Legal Medicine, Coimbra – Portugal

[7]Medicine Faculty of the University of Coimbra – Portugal

[8] Department of Biology, University of Aveiro – Portugal

Genetic identification of biological samples on different types of fabrics with limited amount of DNA, subjected to detrimental conditions and with inhibitors, is a challenge. UV light, heat, microbial degradation and the presence of inhibitors are the major factors which interfere with the analysis of a biological sample.

Our aim was to find out the best DNA extraction method to enable the genetic identification of limited DNA samples with inhibitors, and comprehend the obtained results based on meteorological conditions verified during the sample degradation.

Blood was collected from three donors and blood stains were made in denim, cotton and lycra. Blood stains were dried at room temperature before being placed in clay soil during summer and winter. Blood stains were collected periodically during 90 days. Clay soil was chemically characterized and graphical registers of temperature and rainfall were obtained from the closest meteorological station.

DNA extraction was performed using Chelex method, QIAamp® DNA Investigator kit and DNA IQ™ System kit. DNA was quantified with an ABI Prism®7000 using Quantifiler™ Human DNA Quantification kit. Samples were amplified with AmpFlSTR®IdentiFiler™, AmpFlSTR®MiniFiler™ kits and analyzed in ABI Prism®3130 and ABI Prism®3500 Analyzers.

DNA degradation was much faster during the first 3 days of summer than winter season and varied considerably with temperature and humidity.

The QIAamp® DNA Investigator was the best extraction method, which enabled the recovery of more quality DNA. Complete genetic profiles were obtained for samples buried for 90 days during summer and 7 days during winter.

A rape case with several unknown perpetrators

Bouzga M[1], Valkvae K.L[1], Mevaag B[1]

[1]Norwegian Institute of public health, Department of Forensic Biology

In October 2011, a woman reported a rape claiming to have been assaulted by several unknown perpetrators. The exact time of the incident was unknown as she was intoxicated. The forensic examination was conducted 6-18 hours after the offense. Intimate body samples and her clothing were collected. The exhibits, received for analyzes included her tights, intimate body swabs, and samples from her pants collected by the police. Presumptive test (AP Brentamine) produced positive reaction on all exhibits.

We wanted to investigate to which degree the spermatozoa from all possible contributors were evenly distributed throughout a stain. Furthermore, if an uneven distribution of spermatozoa would be mirrored in the mixture ratios of the EPG's. And finally, whether the profiles of the contributors could be deduced by combining several different mixture ratios.

A total of 60 samples were collected from the tights (crouch area i.e 25 x25 cm) and examined by microscopy and DNA profiling.

All samples contained a large amount of spermatozoa, regardless of the strength of the acid phosphatase reaction. All sperm fractions showed complex DNA-mixtures with minimum four contributors. By using the variations in the mixture ratios and deconvolution the DNA-profile of two of the contributors could be deduced. A database search produced an "offender hit" for one of these profiles. The other contributors in the mixture might be identified by comparisons with reference samples.

DNA transfer during non- contact social interactions

M.Goray[1], R.A.H. van Oorschot[1]

[1]Victoria Police Forensic Services Department, Melbourne, Victoria, Australia

Multiple DNA transfer has increasingly been raised in court as potential means for the presence of the defendants DNA at the crime scene or on a piece of evidence. This has prompted several investigations into DNA transfer under controlled conditions, however little is published about DNA transfer in "uncontrolled" or real life situations.

Here we examine multiple direct and indirect transfer of DNA within a social setting: three participants having a drink together for a period of time while using communal and individual objects.

All the relevant surfaces were cleaned and sampled prior to commencement. The interactions were unscripted and participants remained blind to the aims of the experiment. The events were videotaped by several cameras so all activities including hand to surface interactions were recorded. Afterwards all surfaces of interest were sampled and the generated DNA profiles analyzed in respect to locations, durations and sequences of hand to surface interactions.

In many instances the DNA profile of the last person or the only person to come in contact with the object was the main or the only profile detected. Some surfaces that provided a negative DNA result prior to commencement provided complex mixtures after the event and contained DNA from participants that did not contact the surface directly. In some instances the participants and/or surfaces acted as vectors for foreign DNA transfer, presumably present on the hands or acquired from personal items used during the event.

These results will further aid our understanding of the phenomenon of DNA transfer.

STR and Y-STR genotying of 30-50-year-old semen stains

M. Hara[1], H. Nakanishi[2], S. Takahashi[1], A. Kido[3], K. Saito[2], A. Takada[1]

[1]Department of Forensic Medicine, Faculty of Medicine, Saitama Medical University Saitama, Japan

[2]Department of Forensic Medicine, Juntendo University School of Medicine, Tokyo, Japan

[3]Department of Instruction, Nagoya Isen, Nagoya, Japan

The results of short tandem repeat (STR) genotyping of very old semen stains has become important evidence in long-term unsolved sexual crimes. In this study, we evaluated whether STR and Y-chromosome specific-STR (Y-STR) genotyping of more than 30-year-old semen stains would be useful for gathering forensic evidence.

We used five semen stains stored at room temperature (30 years × 1, 32 years × 2, 46 years × 1 and 50 years × 1). The acid phosphatase test was performed using SM test reagent and the sperm were prepared for microscopy using the Baecchi-stain method. DNA extraction and purification were performed with a EZ1 Investigator Kit, a QIAamp Mini Kit, ISOHAIR, and a DNA Extractor FM kit using a 1 × 0.5-cm piece of gauze. STR genotyping was performed using the AmpFlSTR Identifiler and AmpFlSTR Yfiler kits after DNA quantification using a Human-DNA Quantification kit.

All samples showed positive reactions against the SM test reagent, and we microscopically observed sperm heads in all samples. The quantity of DNA extracted from the 46- and 50-year-old samples was much lower than from the other samples. STR genotyping of the 30, 32, 46, and 50-year-old semen samples detected 15, 15, 12, and nine of 15 loci, respectively, using the AmpF1STR Identifiler kit. Y-STR genotyping detected all 16 loci in all samples. The results suggest that STR genotyping of very old semen stains is useful for gathering forensic evidence.

To destroy snail mail: Is this the sole solution for anthrax contaminated letters?

M. Omedei[1], C. Martino[1], S. Gino[1]

[1]University of Study of Turin, Department of Public Health and Pediatrics, Laboratory of Criminalistic Sciences, Turin, Italy

[2]University of Study of Rome "Tor Vergata", Department of Public Health, Rome, Italy

Suspicious packages, strange addresses on envelopes and/or the presence of particular powders: these are the most popular aspects of letters containing *Bacillus Anthracis.* Since the World Trade Center tragedy, alarmism about chemical or biological attacks is always in force.

The Italian Ministry of Foreign Affairs introduced new procedures to be followed in case suspected anthrax letters are identified (Ministry of Health PROT. 400.3/120.33/4786 of 23/10/2001).

Scientists have to collect samples from surfaces and infectious waste have to be placed in autoclavable bags for decontamination. After the sterilization, mails and packages are burnt thus eliminating every biological trace present on their surface.

As a matter of fact, after sterilization, DNA is still present and can be analyzed for forensic purposes: for this reason, here we report on the importance of preserving sterilized substrates.

We recreated false infected mails with biological traces on their surfaces, sterilized them and, subsequently, we took samples of biological stains and processed them for DNA quantification and typing. We recreate different time conditions consistent with those of the postal service too.

Real-Time PCR and DNA typing showed that, even if sterilization destroys the *bacillus*, human genomic traces still persist and we obtained both complete and partial profiles of samples' donors.

To conclude, the problem of anthrax contaminated letter call for peculiar and standardized procedures; nonetheless, we show that burning evidences after the sterilization process does not appear to be the best solution since there is a loss of biological material which could be decisive for forensic purposes.

Typing of Touch DNA on Crime Scene Evidences By Using Mini STR: A Modeling Study

Miray Aksoy[1], M. Ozlem Kolusayin[2], Itır Erkan[3], Tolga Zorlu[1], Gulten Raimoglu, Ozlem Bulbul[1], E. Hulya Yukseloglu[1]

[1]Istanbul University, Institute of Forensic Science, Cerrahpasa, Istanbul, Turkey

[2]Istanbul University Cerrahpasa Faculty of Medicine Forensic Medicine Department, Cerrahpasa, Istanbul, Turkey

[3]Yeni Yuzyil University Health Sciences Faculty, Topkapi, Istanbul, Turkey

During the crime activity, if the perpetrator only leaves small numbers of skin cells on an item or another human at the scene, and that item is collected as possible evidence, touch DNA analysis may be able to link the perpetrator to the crime scene. Despite the potential DNA transfer by contacts, while on DNA recovery forensic scientists may face problems as contamination or limited amounts of DNA. The objective of the present study is to perform identification by modeling the obtained trace amounts of epithelium DNA from different objects situated in crime scenes. 88 samples were obtained from 4 volunteers ages between 20 and 55. DNA was analyzed using Low Copy Number (LCN) methodology with Mini STR PCR amplification kit and ABI 310 capillary analysis. Obtained DNA quantities were determined that differ between 0,2 – 0,626 µl/mg and mostly touch DNA samples could be successfully typed from surfaces. According to the results, acquirement of the DNA materials shown differences based on the sex/ age of the donor, types of surfaces and shedder index of the perpetrator. LCN profiling known "touch DNA" was developed in 1997 to provide a DNA profile from limited forensic samples (<100 pg) they could have been deposited by a mere touch. The usefulness of this technique will ensure its continued use in forensic investigation further research into DNA transfer and LCN DNA profile interpretation would

increase its evidential value and decrease misconception of DNA profiling methodology. Furthermore statistical analyses will be carried out and interpreted.

Keywords: Touch DNA, Mini STR, CSI

The use of forensic genetic analysis in dental samples for crime scene investigation: a modeling study

Nilüfer Akman Dogan[1,4], M. Ozlem Kolusayin[2], Itır Erkan[3], Tolga Zorlu[1], Gulten Rayimoglu[1], Ozlem Bulbul[1], Ali Volaka[1], Emel Hulya Yukseloglu[1]

[1] Istanbul University Institute of Forensic Science, Cerrahpasa, Istanbul, Turkey

[2] Istanbul University Cerrahpasa Faculty of Medicine Forensic Medicine Department, Istanbul,Turkey

[3] Yeni Yuzyil University Health Sciences Faculty,Topkapi,Istanbul,Turkey

[4] Gebze Institute of Technology, Molecular Biology and Genetics Department, Kocaeli, Turkey

Stability and unique quality of DNA acts an important role on identification of the deceases that are highly composed, corrupted in terms of body integrity, burned or available as skeletal remains. Forensic dental analysis is very useful tool on forensic odontology, disaster victim identification, diagnoses of skeletal remains and burned deceases. Tooth provides a suitable source of DNA since the dental pulp is covered with external agents by resistant tissues that protect DNA from degradation. Our research objectives are to evaluate the tooth samples exposed to various conditions and to identify the tooth samples by genetic techniques. Dental elements (buccal swabs and tooth samples) were collected from 100 donors via various dental clinics. Tooth samples were exposed to sea water /fresh water, buried, kept in room temperature and burned with alcohol. DNA materials from teeth were extracted using QIAamp DNA Investigator kit. Extracted DNA quantities were determined that differ between 0.5-1.62 µl/mg. Buccal swabs were used for control groups and compared with teeth. DNA analysis was carried out with AmpFISTR Identifiler PCR kit on ABI 310 Genetic Analyzer. In our study, the importance of tooth samples acquired from crime scene considered as important DNA sources since of its complex structure were emphasized and the impacts of biological, chemical, physical and environmental conditions on tooth samples were investigated. Furthermore statistical analyses will be carried out and interpreted.

Key Words: STR, Dental Samples, CSI

Circulating cell free DNA of plasma samples for personal identity DNA testing

P. Tasinato[1], E. Seganfreddo[1], M. Montisci[2], G. Basso[1], G. te Kronnie[1]

[1]University of Padova, Department of Child and Women Health, Italy

[2]University of Padova, Department of Molecular Medicine, Italy

Circulating cell free nucleic acids have been detected in peripheral blood specimens of healthy subjects as well as in patients.

With the aim to test the applicability of ccfDNA analysis in human identification, we verified short tandem repeats (STRs) PCR amplification in extracellular circulating DNA.

Materials and methods The study was approved by the ethics committee of the University - Hospital, Padova, Italy (No. 2105P).

2 ml of venous blood in EDTA from 10 men and 10 non-pregnant women.

1 ml of whole blood was centrifuged. The supernatant obtained was transferred to a new 2,5 ml micro tube, re-centrifuged to eliminate platelets in the plasma sample.

ccfDNA was extracted in two replicates from 200 ul of plasma using a QIAamp Mini blood kit (Quiagen, Valencia,). Quantification was performed by real-time PCR (RT-PCR) using the relative quantification with a standard curve method. Albumin (gene) was used as reference. As profile control, 100 ul of DNA from 200 ul for each whole blood sample was obtained with the same extraction protocol.

Genetic human identification analysis was performed by the commercial kit AmpFl STR NGMâ (AB) on the extracted ccfDNA and DNA. For all cases, the STR profile generated from ccfDNA was compared to the matching STR profile generated by DNA obtained from whole blood.

We report complete concordance between amplified alleles from the cell free fractions and the matched DNA samples from whole blood, demonstrating that ccfDNA can be exploited for identification purposes.

Keywords: Forensic Science, DNA typing, circulating cell free nucleic acids; plasma; STR; identification

A deep sequencing investigation of VWA, D16S1156, AME and D8S1179 on cell free DNA of a mother's to be blood

P. Tasinato[1], S. Bresolin[1], G. Basso[1], MT. Gervasi[2], G. te Kronnie[1]

[1]University of Padova, Department of Child and Women Health, Italy

[2]University - Hospital of Padova, Section of Obstetrics and Gynecology, Italy

The profiling of short tandem repeat (STR) loci is currently used in forensic genetics for identification purposes.

We recently demonstrated the possibility to perform DNA profiling for identification purposes on plasma DNA also named cell free DNA.

Our attempt to investigate foetal STR profiles from plasma DNA of pregnant women, carrying also a small proportion of cell free foetal DNA, with traditional STRs amplification and fragments length separation by capillary electrophoresis (CE) failled. Recently, it has been demonstrated that pyrosequencing has exceeding capacity of data generation and discriminatory power.

Here we embarked on a journey of Next Generation Sequencing (NGS) on a DNA specimen from plasma of a pregnant woman. This new approach represents an STR profiling method based on the use of Roche Genome Sequencer Junior 454 Life Science Titanium simultaneously sequencing four STR loci of forensic interest.

We found that 454 STR sequence data out put is composed of maternal and foetal STR sequences.

Influence of the moisture content of forensic swabs on DNA recovery from trace evidence

J Kriehn[1], A Dorn[1], C Schäfer[1], P Wiegand[2], W Voll[1]

[1]Bavarian State Office of Criminal Investigation, Forensic DNA-Analysis, Maillingerstr. 15, 80636 München, Germany

[2] Institute for Legal Medicine, University hospital of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Different types of forensic swabs are used to secure trace evidence for DNA analysis from objects left on crime scenes. Because it remains uncertain how much cellular material is collected by the forensic swab - especially if a stain is not visible such as saliva - two types of forensic swabs (cotton versus viscose) were tested under various conditions.

There are many different parameters influencing the effectiveness of DNA recovery such as the age of the trace, the surface from which the trace is collected, the texture of the swab and the storage time until analysis is performed. But the most important influencing factor seems to be the way in which the trace is collected.

In several experiments we determined which method is the best to transfer the highest amount of cellular material onto the swab. Glass bottles were prepared with a defined amount of saliva and traces were collected in a standardized way. The following factors were investigated regarding the recovery of DNA:

- the effect of the moisture content of the swab
- the pressure during the collection
- the change of contact surfaces on the swab

First results show that the moisture content of the swab has a significant impact on the recovery of the defined amount of DNA.

Touch DNA collection from improvised explosive devices: a comprehensive study of swabs and moistening agents

S. Phetpang[1], T. Kitpipit[1], P. Thanakiatkrai[1,*]

[1]Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

**Corresponding author: pthanakiatkrai@gmail.com*

Improvised explosive devices (IEDs) are used in devastating terrorist attacks worldwide and daily in Thailand. Touch DNA deposited during IED assembly are subjected to intense heat and pressure, resulting in rare events of usable DNA profiles obtained from real casework. No study has simultaneously evaluated both swab types and moistening agents for touch DNA collection from substrates encountered in IED evidence. In this study, we investigated the effects of swab types and moistening agents on DNA collection from PVC, a common IED substrate. A full factorial design using seven swab types (two forensic cotton swabs, two medical cotton swabs, two forensic nylon swabs, and one foam swab) and six moistening agents (sterile water, phosphate-buffered saline, ethanol, sodium dodecyl sulfate, isopropanol, and lysis buffer) was employed (42 total combinations). Using buffy coats, we found that DNA recovery depended on both swab types and moistening agents ($p<0.05$). The optimal method recovered significantly higher DNA amount from real IED cases compared to the standard Royal Thai Police method. Percentages of full and high partial also increased. Our results changed the standard operating protocol of the Thai police. Other commonly found substrates from IED cases are being investigated to maximize the evidential value obtained from touch DNA on IEDs.

Funding: Graduate Studies Research Grant, Prince of Songkla University

Keywords: touch DNA; swab; moistening agent; bombing cases

Comparative study on the effects of reduced PCR reaction volumes and increased cycle number on the sensitivity and the stochastic threshold of the AmpFlSTR Identifiler® Plus kit.

M.W.Bessekri[1], F. Agsous[1], S. Lazreg,[1] K. Daho[1], R Bucht[2], V Fuller[2]

[1]Department of Biology - Institute Nationale de Criminalistique et Criminologie - Gendarmerie Nationale – Algeria

[2]US Department of Justice - International Criminal Investigative Training Assistance Program – Algeria

A variety of commercial *STR kits* are available to the *forensic* DNA community for quick and reliable DNA results. The high cost of kits and increasing number of DNA samples submitted make it critical to implement cost-effective improvements in the use of amplification kits.

The purpose of this study is to compare the performance of reduced volume PCR reactions with the Identifiler Plus kit and to investigate the influence on the sensitivity and stochastic occurrences under standard and increased thermal cycling conditions. The study assessed three final volumes of 25µL, 10µL and 5µL, where the reagents and template DNA were kept in the same proportions. Seven serial two-fold dilutions of the positive DNA control (9947A) were amplified in five replicates for each reaction volume tested, starting with the optimal concentration of 0.04ng/µL. In addition, two cycling protocols (28 and 29 cycles) were tested with each reduced volume and each DNA dilution.

Among the three volumes, sensitivity and stochastic effects appeared to be unchanged within each cycling condition. Under the 29 cycle condition, the sensitivity was higher than under standard cycling conditions but there was also a consequent increase in background noise and stochastic threshold. The limitation of reducing volumes appears to be mainly due to the challenge of small volume manipulation. As such, a 10µL final volume proved to be both efficient and economical while simultaneously significantly decreasing the amount of evidence consumed.

A comparative study of the efficiency of two protocols of DNA extraction from bones

S. T. G. Ferreira[1]

[1]Instituto de Pesquisa de DNA Forense – Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil

DNA laboratories use different protocols for DNA extraction from bones according to the kind of samples, quality and degree of degradation of bones and available technologies, among other variables. The aim of this study was to compare the efficiency of two protocols of DNA extraction from bones in different states of preservation. We compared modified organic phenol-chloroform protocol with the standard extraction protocol described in Edson et al, 2004. For this study, 20 bone samples from femurs were selected from caseworks. 13 samples were selected from human remains buried between the years 1993 and 2010 (exhumed during 2009 and 2012) and 7 samples were selected from not buried skeletal remains found outside between the years 2002 and 2010. Each bone sample was prepared and then powdered in a cryogenic mill (CertiPrep 6750 Freezer Mill Spex) and tested in both protocols. For each sample, 2 grams of powdered bone were used**.** All samples were incubated overnight, organic extraction was performed and DNA extracted was purified and concentraded with Centricon 100 Centrifugal Filter Units (Millipore). Quantifiler Duo Kit (Life Technologies) was used for quantification and Identifiler and MiniFiler Kits (Life Technologies) were used for amplification. The efficiency of the protocols was compared according to the amount of DNA yield from each method and to the quality of DNA typing results. In this study, modified organic phenol-chloroform protocol yielded higher amounts of DNA and better DNA typing results (STR profiles) than the standard protocol in Edson et al, 2004.

Cartilage: an alternative source of samples for DNA typing in disaster victim identification (DVI). A comparative study

S. T. G. Ferreira[1], R. G. Garrido[2], K. A. A. Paula[1], A. Moraes[1]

[1]Instituto de Pesquisa de DNA Forense – Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil

[2]Instituto de Pesquisa e Perícias em Genética Forense – Polícia Civil do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

In disaster victim identification (DVI) the kind and quality of source of samples for the DNA testing will play a decisive role in victims' identification. The aim of this study is to demonstrate that cartilage is an important alternative source of sample for DNA testing in DVI. In January 2011, the biggest natural disaster (918 deaths) ocurred in Brazil due to floods and mudslides in the State of Rio de Janeiro. In this study, we compared DNA testing of 25 bone samples collected from lower limbs (foot phalanges and metatarsals) of 25 intact bodies with 25 cartilage samples collected from undamaged joints (knees) of the same 25 intact bodies. DNA testing of bone samples was performed in 2011, during the victims' identification work. All cartilage samples were stored in January, 2011, and were tested only in March, 2013, two years after the incident, for this comparative study. Sample collection was performed with disposable equipment. Modified organic phenol-chloroform was used for DNA extraction. Plexor HY Kit (Promega) was used for real-time quantification and Identifiler Plus (Life Technologies) and PowerPlex 16 and Fusion (Promega) Kits were used for amplification. In both cases, bone samples tested in 2011 and cartilage samples tested in 2013 had high amounts of DNA yields and excellent DNA typing results as it will be shown in this study. Cartilage is easy to be collected in mass fatality incidents and proved to be an excellent kind of sample for DNA typing in DVI, even long time after its collection.

DNA typing of trace DNA recovered from different regions of sandals found in a homicide crime scene investigation: a comparative study

S. T. G. Ferreira[1], K. A. A. Paula[1], A. V. Moraes[1]

[1]Instituto de Pesquisa de DNA Forense, Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil

Trace DNA typed from evidences found in crime scenes may help investigations and identify perpetrators and victims. The aims of this study were to compare data provided of trace DNA recovered from different regions of a pair of sandals found in a real homicide crime scene and to demonstrate the importance of DNA trace evidence in criminal investigations. A pair of sandals, made with synthetic material (rubber), found in a homicide crime scene, was collected by the investigators and stored at room temperature during five months. After this period of time, the sandals (the right and the left one) were sent to DNA laboratory and were carefully examined. Each sandal were swabbed in parts, varying from regions of more possible contact and abrasion of skin cells (ankles, toes and straps) to regions with less possible contact (middle surface of the sandals). Modified organic phenol-chloroform protocol was used for DNA extraction. Plexor HY Kit (Promega) was used for real-time quantification and Identifiler Kit (Life Technologies) was used for amplification. The amount of DNA recovered from trace DNA varied from region to region in the different parts of the sandals. However, DNA typing results were obtained from all swabbed regions. A suspect of the murder was DNA typed and his DNA profile matched with the one recovered from the sandals. Trace DNA typed proved decisive in the reconstruction of the crime scene and in the identification of the murder, demonstrating the importance of trace DNA in helping investigations.

<Insert S.T.G Ferreira abstract>

Validation of Copan dual 4N6FLOQSwabs™ for original sample record retention

Castriciano S[1], Squassina A[1], Gervasoni A[1]

[1]Copan Italia SpA. Brescia, Italy

References and further reading may be available for this article. To view references and further reading you must purchase this article.

Original sample retention from evidence or paternity testing is a requirement. Currently a swab portion is shaved for testing and the leftover is stored as record. Copan is producing dual 4N6FLOQSwabs™ (D4N6FS), consisting of 2 identical swabs attached to the same handle in a plastic tube. Each swab has a 20mm breaking point to facilitate one swab testing and storing the other in the original tube. D4N6FS are available for Genetics and Crime scene (CS), the latter treated with a reagent to prevent bacterial prolypheration. The aim of this study was to evaluate the D4N6FS for: sample recovery ability, ease use and practicality and comparable sample amount collection from both swabs on the same buccal sample or blood trace.

Using Copan guides were collected from donors: buccal swabs with Genetics D4N6FS or dried blood traces with CS D4N6FS. Dual swabs were placed into their own tubes and tested single as well as the references. Nucleic acid was extracted with the PrepFiler® , quantified with the Quantifiler® and profiled with the Identifiler Plus® (by Life Technologies).

Similar quantities of DNA were obtained from each swab tested separately among all buccal or blood traces. Data demonstrated that D4N6FS: recover enough sample to obtain a full STR profile; are user-friendly and practical for sample testing and record retention; collect comparable amount from the same sample by each of 2 swabs. Copan Genetic and CS D4N6FLOQSwabs facilitate sample retention for retesting without manipulations

Copan 4N6FLOQSwabs™ collection guidelines are improving evidence investigations

Castriciano S[1], Gervasoni A[1], Squassina A[1], Bailey J.A.[2]

[1]Copan Italia Spa, Brescia, Italy

[2] Minnesota State University, Mankato, MN, USA

References and further reading may be available for this article. To view references and further reading you must purchase this article.

Copan 4N6FLOQSwabs™ (4N6FS) are innovative devices for crime scene evidence collection. 4N6FS, consisting of nylon fiber strands attached to molded plastic, have high hydrophilic activity that allows efficient sample collection and release. 4N6FS, produced only by profiled staff, are ETO treated and DNAse, RNAse and human DNA free. 4N6FS are specially produced for Genetics and Crime scene, the latter treated with a bacteria-static agent to prevent microbial prolypheration. The 4N6FS are available in a short format in a wider tube; therefore the tip is never in contact with the tube's inner surfaces. The aim of this study was to develop proper collection guides to improve DNA recovery from crime scenes.

Saliva and blood traces were prepared on different surfaces. Samples were collected from each of the prepared surfaces with 4N6FS using different methods. Saliva and blood from donors were used as references. Nucleic acid was extracted with the PrepFiler® quantified with the Quantifiler® kit on the 7500 PCR system and profiled with the Identifiler Plus® kit (by Life Technologies).

Quantity of DNA obtained with all collection procedures was compared to the references. The collection procedure that detected similar amounts of DNA as the reference samples was used to prepare the collection guides. The guidelines were assessed by several crime scene labs and lastly revised by a USA University Professor of Political Science and Law Enforcement. Copan 4N6FLOQSwabsTM collection guidelines are improving evidence investigation, shared and available for all the users.

Evaluation of five DNA extraction systems for recovery of bone DNA

S. Iyavoo[1,2], S. Hadi[1], W. Goodwin[1]

[1]School of Forensic and Investigative Sciences,University of Central Lancashire, United Kingdom

[2]Forensic Division, Department of Chemistry Malaysia (KIMIA), Ministry of Science and Technology, Malaysia

Isolation of DNA from skeletonised human remains can be problematic. In addition to DNA degradation, enhanced by high temperature and humidity, there are often potent PCR inhibitors present within the samples. It is therefore important to extract the maximum amount of available DNA whilst removing any PCR inhibitors that may be present.

Five DNA extraction systems were assessed for their DNA extraction efficiency on samples of fresh pig bone. Four commercially available silica-based extraction kits: ChargeSwitch® gDNA Plant Kit (Life Technologies), DNA IQTM System Kit (Promega), DNeasy® Blood & Tissue Kit (Qiagen) and PrepFiler® BTA Forensic DNA Extraction Kit (Life Technologies) and a conventional phenol-chloroform method were used according to the manufacturers' instructions and published methods. Extracted DNA samples were quantitated with GoTaq® qPCR Master Mix (Promega) using Applied Biosystems® 7500 Real-Time PCR System.

Phenol-chloroform extraction produced higher yields of DNA than the silica-based extraction methods. Among the silica-based extractions ChargeSwitch® gDNA Plant Kit (Life Technologies) showed the highest DNA recovery.

Screening and identification of tissue-specific methylated DNA markers for forensics

S. Yi[1], L. Ma[1], D. Huang[1]

[1]Department of Forensic Medicine, Huazhong University of Science & Technology, Wuhan, China

Tissue identification of biological materials encountered at crime scenes can bring important information in some cases. Current visual, catalytic, enzymatic, and immunologic tests for tissue identification are applicable only to a subset of samples, might suffer limitations such as low specificity and sensitivity, impact from environment and operators. An alternative approach is based on RNA; however, RNA is not as stable as DNA. Tissue-specific differentially methylation makes it possible to identify tissue based on analysis of DNA. We designed a test to screen and identify tissue-specific differential DNA methylation markers for tissue identification.

DNA samples from blood, saliva, semen, vaginal epidermis, skin epidermis and muscle were analyzed by methylation sensitive represent difference analysis. Tissue specificity of methylation profiles of the screened fragments was evaluated by Sequenom Massarray® quantitative analysis of methylation followed by methylation specific PCR.

Nine blood specific methylated markers and eleven muscle specific methylated markers were successfully isolated. The blood and muscle tissues were successfully identified by assay of the isolated specific methylated markers in all seventy cases. The result indicated that more tissue-specific methylation markers can be selected from genome for forensic application by proper methodology and methylation-based tissue identification will be another viable DNA analysis method that could be put into forensic practice in future.

Age estimation using quantification of sjTRECs for forensics in Korean population

Cho. S.[1], Lee. HY.[1], Kim, K.[1], Lee. J.[1], Lee. SD.[1,2]

[1]Department of Forensic Medicine, Seoul National University College of Medicine, Seoul, Korea

[2]Institute of Forensic Medicine, Seoul National University College of Medicine, Seoul, Korea

Age estimation using biological samples remained in crime scene may provide valuable information about the criminals. Several methods for estimation of age have been introduced, and these include odontological or skeletal analysis, age-dependent accumulation of mtDNA deletions and shortening of telomere length. Recently, a new promising method using the measurement of a DNA product excised during TCR rearrangement (sjTREC; signal-joint T cell receptor excision circle) was reported and it was demonstrated the much improved accuracy. We have modified the previously reported method by making the amplicon short and tested it for samples obtained from 172 Korean people ranging from 16 to 65 years old. Negative correlation between sjTREC levels and age was observed with r=-0.799, and the standard error of the estimate was 8.63 years. Additionally, the applicability of tissues other than blood including thymus and spleen were checked whether they could be applicable for the above immunological age test. In conclusion we have provided the guideline for the age estimation using the modified method in Koreans with advanced sensitivity, and have confirmed the extensibility of this test for the samples other than blood, including thymus and spleen.

The fund for research was supported by National Forensic Service.

Is the acoustic prothesis the father of the stamp?

S. Turrina [1] , S. Caratti [1], D. De Leo [1]

[1] Department of Public Health and Community Medicine, Institute of Legal Medicine, Forensic Genetic Laboratory, University of Verona, Verona, Italy

We describe an unusual case of deficient paternity testing, requested by the heirs, the wife and two legitimate daughters, on a father and his putative son, deceased respectively in 2007 and 2011. The analysis was resolved on two personal items of the defuncted: a postcard stamp 17 years-old, presumably sent by the putative son, and an acoustic prothesis belonged to the alleged father.

Fifteen autosomic short tandem repeats (STRs) from the commercial kit AmpFlSTR Identifiler® kit (Applied Biosystems) were analyzed on the acoustic prothesis and the mother-daughters trio, giving a probability of paternity (W) of 99.9999% and therefore confirming that the father owned the prothesis. Analysis of both autosomal and Y-chromosomal STRs by means of Powerplex® Fusion System and Powerplex® Y23 System (Promega, Madison, WI) was carried out on the two personal belongings.

The obtained probability of paternity value W > 99,998 from autosomal STRs and the presence of an identical Y-chromosomal haplotype shared between the two males confirmed the genetic compatibility of the alleged father and his son.

Evaluation of PowerPlex® Fusion System on samples from forensic casework

S. Turrina [1], S. Caratti [1], D. De Leo [1]

[1] Department of Public Health and Community Medicine, Institute of Legal Medicine, Forensic Genetic Laboratory, University of Verona, Verona, Italy

A newly-commercialised STR amplification kit, PowerPlex® Fusion System (Promega, Madison, WI) was evaluated on forensic casework samples and paternity testing. This kit is a 24-loci multiplex including CODIS and European Standard Set (ESS) core loci, plus D2S1338, D19S433, Penta E, Penta D markers, Amelogenin for gender discrimination and locus DYS391 for identification of potential null alleles results for Amelogenin.

Difficult forensic casework samples included teeth, fingernails, mummified skin, formalin fixed paraffin-embedded (FFPE) tissues, buried scheletal muscle and DNA samples collected from various objects (sunglasses, clock, glass, sex toys). Samples were extracted with both QIAamp DNA Mini kit and QIAamp DNA Investigator kit (Qiagen) and subjected to 28-32 cycles of PCR amplification. Fragments were analyzed on an ABI Prism 3130 Genetic analyzer (Life Technologies) with software GeneMapper ID-X v1.2

On average, more than 9 loci could be reliably typed in all forensic casework samples. Furthermore complete drop-out of loci greater than 250-300bp, including DYS39, was observed in highly degraded samples. However, overall sensitivity of the system in typing difficult samples with low amounts of template and in presence of inhibitors was high. In paternity samples when more than 0.5-1ng of template DNA were amplified, profile interpretation was not straightforward due to allelic drop-in and peak imbalance. Interestingly, a rare tri-allelic pattern at locus Penta E was detected in a single individual. Although further validation studies are required, this STR kit represents a valid tool for the analysis of challenging forensic samples.

Bone sampling strategy and success rates for missing persons DNA identification

S. Amory[1] , M. Sarajlic[1] , R. Huel[1], M. Vennemeyer[1], A. Bilic[1] , A. Rizvic[1] , T. Parsons[1]

[1]International Commission on Missing Persons, Sarajevo, Bosnia Herzegovina

The ICMP conducts large scale DNA identifications on skeletal samples from mass graves or mass disasters. Traditionally teeth and long bones are the samples of choice for DNA based identification whereas smaller bones are usually used only as a last resort. However, often remains are commingled within the same grave or amongst separated burial sites thus requiring the testing of smaller, more challenging samples, to assist with the re-association of remains.

In this study we compiled results obtained from more than 10,000 bones collected from various grave sites related to the Western Balkans Conflict. These bones were buried in diverse environments such as mass graves, caves, under water or surface sites. DNA was extracted using a full demineralization protocol coupled to a silica based purification method. This recently improved extraction method has increased the range of sample types which provide reliable results.

This data set allowed us to determine DNA typing success rates from various skeletal elements, but also to assess the influence of the taphonomic conditions on the DNA preservation. As expected teeth, femur and tibia showed high success rates. However, smaller elements not usually tested also proved to be good samples for DNA testing. Other long bones such as humerus, radius and ulna were found to be poor choices.

These results obtained from a large sample set were used by the ICMP to refine the sampling strategy especially for commingled cases for which the availability of skeletal element is limited and the sample selection is critical.

DNA Profiling of Disaster Victim Identification in Trenggalek Shipwreck Case

T Dhanardhono[1], N Wulandari[2], SKL Bhima[1], HJ Ahmad[2], PT Widodo[2]

[1]Faculty of Medicine Diponegoro University, Semarang, Central Java, Indonesia

[2]DNA Laboratory Indonesian National Police, Jakarta, Indonesia

Trenggalek shipwreck was consider to be an open disaster. DVI management deal with many conditions such as its major location in Indian Ocean, scattered remains in different places, and the unknown passengers manifest. More than 200 people were estimated as the victims. Seawater environment become the main concern, i.e in the evacuation and the impact on post mortem. But, especially in its high saline concentration influence's to DNA. This paper is subjected to evaluate the result of DNA analysis, in the context of its DNA profiles feature and the detectability of Identifiler Plus loci.

Among 103 remains found, 74 DNA samples were able to be extracted from Achilles tendons and subjected to STR DNA typing. Extraction methods utilizing Chelex procedure. Quantification using Quantifiler Human DNA. PCR reaction were setted into ABI prism 7500 DNA thermal cyclers. Identifiler Plus marker were used to yield DNA profile. Amplicons were run into capillary electrophoresis 3130xl DNA sequencers and analyzed with the Gene mapper 3.2 softwares.

The deceased body was found within days and weeks. Among 74 bodies, male victims identified as much as 48 numbers, while 26 remains identified as female. Amelogenin marker show a constant result to distinguish sex. Full DNA profiles were yielded in 46 victims. Several loci established 100% detectability, such D8S1179, D3S1358, TH01, D5S818, FGA and AMEL. Profiling a DNA samples derived from seawater may encounter various environment condition which lessen its quality.

Key words : DNA profiling, DVI

DNA extraction method from teeth using QIAcube

V. Pinchi[2], M. Focardi[2], D. Martinelli[2], G.A. Norelli[2], I. Carboni[1], A. Gozzini[1], C. Romolini[1], F. Torricelli[1], U. Ricci[1]

[1] AOU Careggi, SOD Diagnostica Genetica, Florence, Italy

[2] AOU Careggi, Dipartimento di Anatomia Istologia e Medicina Legale, Servizio Medicina Legale, Florence, Italy

The use of semi-automatic extraction from forensic samples facilitates both contamination control and method reproducibility. However, when an accredited ISO17025 forensic laboratory starts to use a new analytical system, an internal validation procedure is needed before processing forensic samples. Here we show validation results obtained by introducing QIAcube technology for DNA extraction from teeth that enables seamless integration of automated low-throughput sample preparation into the laboratory workflow. Purification steps, in association with QIAamp DNA Investigator Kit, are fully automated thus preventing cross contamination.

A total of 22 teeth stored for 4-5 years after removal and 8 teeth stored for one-two months at room temperature underwent to DNA extraction by using this system. Teeth were previously decontaminated and endodontic access to pulp chamber was performed according to technique described by Pinchi et al (2011)[1]. A blood reference of the teeth donors was also collected. After DNA extraction all samples were quantified by using Quantifiler® Duo DNA Quantification Kit. Total DNA ranging between 0.2 and 350 ng in 60 µl of TE buffer with no inhibitors presence in any sample. PCR amplification by using The obtained results confirm the robustness and reproducibility of the method of extraction with technology QIAcube.

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Genetic identification of biological samples buried in clay soil during Summer and Winter seasons

V. Bogas [1], M. Carvalho [1,2], L. Souto [2], M.J. Anjos [1,2], M.F. Pinheiro [2,3,4,5], F. Corte-Real [2,6,7]

[1]Forensic Genetic Service, Centre Branch, National Institute of Legal Medicine, IP, Coimbra – Portugal

[2]CENCIFOR, Forensic Sciences Centre, Portugal

[3]Forensic Genetic Service, North Branch, National Institute of Legal Medicine, IP, Porto - Portugal

[4]Faculty of Health Sciences of University Fernando Pessoa, Porto – Portugal

[5]Faculty of Biomedical Sciences "Abel Salazar", Porto - Portugal

[6]Centre Branch of National Institute of Legal Medicine, Coimbra – Portugal

[7]Medicine Faculty of the University of Coimbra – Portugal

[8] Department of Biology, University of Aveiro – Portugal

Genetic identification of biological samples on different types of fabrics with limited amount of DNA, subjected to detrimental conditions and with inhibitors, is a challenge. UV light, heat, microbial degradation and the presence of inhibitors are the major factors which interfere with the analysis of a biological sample.

Our aim was to find out the best DNA extraction method to enable the genetic identification of limited DNA samples with inhibitors, and comprehend the obtained results based on meteorological conditions verified during the sample degradation.

Blood was collected from three donors and blood stains were made in denim, cotton and lycra. Blood stains were dried at room temperature before being placed in clay soil during summer and winter. Blood stains were collected periodically during 90 days. Clay soil was chemically characterized and graphical registers of temperature and rainfall were obtained from the closest meteorological station.

DNA extraction was performed using Chelex method, QIAamp® DNA Investigator kit and DNA IQ™ System kit. DNA was quantified with an ABI Prism®7000 using Quantifiler™ Human DNA Quantification kit. Samples were amplified with AmpFlSTR®IdentiFiler™, AmpFlSTR®MiniFiler™ kits and analyzed in ABI Prism®3130 and ABI Prism®3500 Analyzers.

DNA degradation was much faster during the first 3 days of summer than winter season and varied considerably with temperature and humidity.

The QIAamp® DNA Investigator was the best extraction method, which enabled the recovery of more quality DNA. Complete genetic profiles were obtained for samples buried for 90 days during summer and 7 days during winter.

Following the transfer of DNA: how far can it go?

V.J. Lehmann[1,2], R.J. Mitchell[1], K.N. Ballantyne[2], R.A.H. van Oorschot[2]

[1]La Trobe University, Melbourne, Victoria, Australia

[2]Victoria Police Forensic Services Centre, Melbourne, Victoria, Australia

DNA transfer is an issue that is becoming ever more important, due to DNA analytical techniques detecting smaller amounts of DNA found at crime scenes, and leads to questions relating to 'how it got there,' rather than 'who it came from.' Whereas primary and secondary DNA transfer have been studied, tertiary and subsequent transfer steps have been much less focused on. This study aims to measure how detectable a target DNA source is after multiple transfer events. Factors influencing DNA transfer such as the type of biological substance and substrate, freshness of deposit, and the presence of background DNA, will be considered.

In preliminary studies, 15µL of blood was deposited on a cotton substrate and transferred whilst both when wet, and when dry, to a second cotton substrate, which was then transferred to a third, and so forth, until contact with a sixth substrate. Transfer of wet blood showed that a full profile was obtainable after multiple transfer events. However, with dry blood, a full profile was not obtained after the first transfer step. Studies incorporating other factors are currently in progress.

The results of this study will contribute to a greater understanding of the tertiary and subsequent transfer events of DNA which will allow for better evaluation of the likelihood of alternative scenarios explaining why an individual's DNA was at the scene of the crime.

A comparative study of two extraction methods routinely used for DNA recovery from postcoital samples

T. Mudariki[1], H. Trimula-Pallikarana[1], L. Ives[2], S. Hadi [1], W. Goodwin[1]

[1]University of Central Lancashire, Preston, UK

[2]Key Forensic Services, DNA Division, Norwich, UK

In sexual assault cases DNA profiling of spermatozoa can be of critical importance. Most methods use differential extraction of the spermatozoa to separate it from the female component. Here we have compared two commercially available differential extraction methods, the QiAamp® DNA mini kit (Qiagen) and Differex™ with the DNA IQ® System (Promega) to evaluate extraction efficiency. Simulated postcoital samples were prepared using buccal cells

from a female donor and spermatozoa from three male donors. A dilution series ranging from neat semen to a 1:1500 dilution (semen:dH2O) was prepared and mixed with an equal volume of saliva from a female donor. Extraction efficiency was assessed using DNA concentration measured with NanoDrop 2000, Quantifiler® Human DNA Quantification Kit and Plexor® HY System and profiling efficiency using SGM Plus, PowerPlex® ESI 17 and PowerPlex® Y. Statistical analysis was carried out using Wilcoxon Rank Test and Randomisation in R, which is a robust model making no assumption of the distribution of data. Based on the amount of DNA extracted and the amplification of autosomal and Y chromosome markers we found no significant differences between the performance of the two kits. However, the processing time taken with the Differex™ System was about half than that of the QiAamp® DNA mini kit and involved fewer liquid transfers.

Genotyping of Fetal Hypermethylated RASSF1A sequence in Maternal Plasma: Implications for Noninvasive Prenatal Paternity Testing

X.L. Ou, H.Y. Sun

Faculty of Forensic Medicine, Zhongshan Medical School, Sun Yat-sen University, Guangzhou, Guangdong, People's Republic of China

Routine prenatal paternity analysis employs invasive tests that carry a risk of miscarriage. As an alternative, we attempted to amplify the fetal SNP alleles within the hypermethylated fetal RASSF1A sequence in maternal plasma.

Methylation status of the RASSF1A sequences were screened in 5 paired placental tissue and maternal blood cell DNA samples, using conventional PCR after digestion with BstUI, a methylation-sensitive restriction enzyme. Hypermethylated RASSF1A sequences in the maternal plasma were further detected from 45 pregnant women and 10 non-pregnant women. ACTB gene was detected as a control to confirm complete enzyme digestion. Direct sequencing was used to determine the SNP genotypes within the RASSF1A amplicons. At last, we applied this RASSF1A genotyping assay to routine paternity testing samples.

The RASSF1A sequence was found to be hypermethylated in placental tissues while hypomethylated in maternal blood cells. Hypermethylated RASSF1A sequences were detectable in the plasma of all 45 pregnant women while absent in the 10 nonpregnant women. The SNP genotype of the hypermethylated RASSF1A in maternal plasma was identical to the fetal tissue, including the studied prenatal paternity testing cases, thus confirming its fetal origin. In conclusion, this study demonstrated the potential utility of fetal epigenetic marker containing informative SNP for prenatal paternity testing.

Development of DNA methylation markers for monozygotic twin discrimination

Y.S. Kim[1,2], J.L. Park[1,2], S.Y. Kim[1,2], H.S. Yoo[1], J.H. Kim[2,3], H.C. Lee[4], K.M. Woo[4]

[1]Medical Genomics Research Center, KRIBB, [2]Department of Functional Genomics, University of Science of Technology, [3]Biomedical Proteomics Research Center, KRIBB, 125 Gwahak-ro, Daejeon, Republic of Korea, [4]DNA Forensic Division, Supreme Prosecutor's Office, 157 Banpodae-ro, Seocho-gu, Seoul, Republic of Korea

In a forensic science, Short Tandem Repeat markers are useful in identifying individuals found at crime scenes, but they can't be used to distinguish genetically identical monozygotic (MZ) twins. In this regard, epigenetic changes such as DNA methylation or histone modifications have a great potential to be used for discriminating monozygotic twins. Indeed, 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. Here, we investigated genome-wide differences in DNA methylation among 18 paired MZ twins using Illumina's Human Methylation 450K array. From more than 480,000 CpG sites, we first selected several hundreds of Differentially Methylated Regions (DMRs) for each twin pair. Next, we selected 35 candidates by searching for recurrent DMRs among the 18 paired MZ twins. We then built a decision tree to discriminate the 18 paired MZ twins, and finally selected 3 DNA methylation markers. Classification accuracy of the combined three markers on the original data set was 90%, suggesting that DNA methylation can be a promising marker to discriminate MZ twins. We are now validating the selected markers in an independent set of 20 paired MZ twins by pyrosequencing. In conclusion, DNA methylation is a good source for developing molecular markers for forensic discrimination of monozygotic twins.

Forensic approach to analyzing rape cases

Z. Jakovski[1], R. Jankova[1], A. Duma[1], B. Janeska[1], G. Pavlovski[1], D Marjanovic[2]

[1]Institute of forensic medicine, criminology and medical deonthology, School of Medicine, Uni. „St's. Cyril and Mdethody", Skopje, Republic of Macedonia

[2]INGEB, Sarajevo, Bosnia and Hercegovina

With large number of criminal acts, such as rape and murder, identification of the perpetrator is very difficult to determine. The cause is the lack of sufficient quantity of biological traces provided as material evidence, or the biological material is decomposed and no result can be obtaind by using other identification methods. Thus the case remains unsolved.

The purpose of this research is to establish a reliable method for detecting semen presence in rape cases and to get DNA profile from the perpetrator of a crime.

Vaginal swabs were taken using cotton swabs during gynecological examination or autopsy in 21 cases. The chemical detection of semen presence was performed using Phospathesmo Kits. The DNA exctraction was performed using QIAAamp® DNA Mini Kit. The amplification was performed using AmpFistrIdentifiler Kit and AmpFistrYfiler Kit. The electrophoresis was performed using 310 ABI squenator.

Results indicate that DNA profile was obtained in 4 cases where chemical tests did not prove semen presence using Phospathesmo Kits. In one case, neither semen presence was chemically proven nor DNA profile was obtained for autosomal STRs, but a profile for Y-STRs was obtained. Our analyses indicate that when the victim's body is examined within the first few hours or the first day, a genetic profile of the perpetrator of the criminal act is obtained. Besides using autosomal STRs, we recommend Y-STRs to be used in all rape cases, too, thus separating the male from female profile, and also the male kinship relatedness in cases of incest could be followed, the rape performed by several blood-related men or similar.

Theme 13: Non-human forensic DNA analysis

Development of an STR Multiplex for the Identification of Pigs (Sus scrofa)

YC. Lin[1,2], H.M. Hsieh[3], J. Chun-I Lee[4], C.T. Hsiao[5], D.Y. Lin[6,7], A. Linacre[8], L.C. Tsai[3]*

[1]Department of Graduate Institute of Biotechnology, National Chung Hsing University, Taiwan ROC.

[2]Taichung City Government Police Department, Taichung City 40758, Taiwan ROC.

[3]Department of Forensic Science, Central Police University, Taoyuan 33334, Taiwan ROC.

[4]Department of Forensic Medicine, National Taiwan University, Taiwan ROC.

[5]New Taipei City Police Department, New Taipei City 22005, Taiwan ROC.

[6]Livestock Research Institute, Council of Agriculture, Tainan City 71246, Taiwan ROC.

[7]Department of Life Sciences, Cheng Kung University, Tainan City, 701, Taiwan, R.O.C.

[8]School of Biological Sciences, Flinders University, Adelaide 5001, Australia.

We report on the establishment of a novel STR multiplex using 13 tetra-nucleotide STR loci and the amelogenin marker for the forensic identification of pigs and pig products. These loci are novel to this species and represent a significant advance compared to the dinucleotide repeats used currently; this is in accordance with a recent ISFG Commission.

We also prepared an allelic ladder to assist with accurate genotyping. Genotypes and allele frequencies were generated based on 273 samples from 13 pig breeds for all 13 loci. Using these data genetic variation was determined using standard tests (this included Na, Ne, Ho, He, Pd and PE) for each STR locus and for each breed. Based upon the 273 samples in this study, the CPd and CPE_{trio} of the 13 STR loci were found to be greater than 0.9999 and 0.9996 respectively. The CPP_{trio} can reach greater than 99.9999 % for paternity testing using data from one putative offspring and both parents.

As part of the validation study, blind trial testing resulted in valid and expected results for all 14 loci. Phylogenetic analysis revealed that European domestic breeds clustered in a single clade whereas the Asian breeds showed multiple clades. A case example demonstrates the utility of this test from which we demonstrate that this novel multiplex is valuable in pig individualization, parentage testing, and phylogenetic studies for forensic applications.

Soil sample metagenome NGS data management for forensic investigation

L. Lilje[1], T. Lillsaar[1], R. Rätsep[1], J. Simm[1], A. Aaspõllu[1]

[1]Tallinn University of Technology, Tallinn, Estonia

Soil analysis is a valuable resource in forensic investigation. Classical forensic soil analysis involves examination of its physical characteristics and chemical composition, such as soil type, colour, particle size, shape, pH, elemental, mineral and organic content. However the limited variability of these parameters is not always allowing adequate discrimination between soil samples. As soil supports extreme diversity of microorganisms and eukaryotic communities, microbiological approaches have been proposed. Several molecular approaches for microbial DNA profiling are available, however there is lack of published data of implementation of the next generation sequencing (NGS) approaches for forensic soil analysis.

The aim of the current study is elaboration of criteria for soil metagenome data management and database searching.

We used our previously sequenced collection 11 samples collected from different environments (forests, fields, grasslands, etc) with different flora. The single sample collection includes 9 soil samples per one sampling area (30 x 30 m) spaced by 15 m. We concentrated currently on 18S rRNA gene V2-V3 region for fungi and SSU rRNA region for arbuscular mycorrhizal (AMF) fungi, which has been sequenced by Roche/454 platform.

NCBI BLASTN analysis revealed altogether 2983 AMF matches and 8997 18S matches. Several data filtration approaches were used for data management (5 to 9 common sequences per sampling area as well as sequence IDs) for model building. Our results have broad impact; however additional studies and cooperation between soil scientist, molecular ecologists and forensic scientists are required to be able to implement these novel techniques routine forensic practice.

Border biosecurity and the use of barcode techniques for species identification in the ornamental fish trade: The case of the South American freshwater pufferfishes

C.R.L. Amaral[1,2], P.M. Brito[2], D.A. Silva[1], E.F. Carvalho[1]

[1]Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[2]Laboratório de Ictiologia, Tempo e Espaço, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

The international aquarium trade reached an annual trade volume estimated at U$15-25 billion, being by far the largest exchange route for several exotic plant, invertebrate, and vertebrate species. In this sense, the poorly regulated international trade, mainly related with ornamental fishes, is a source of major risks to both biodiversity and local economy due to the influx of exotic species and pathogens. The species identification still is one of the major obstacles for efficient law enforcement by the border security officials and reliable tools, protocols, and databases for species identification are still needed. Here we used a barcoding approach firstly to verify the usefulness of the technique applied for Tetraodontidae species, followed by its application to investigate the case of the freshwater pufferfish *Colomesus*, one of the South American species commonly found on the aquarium fish trade.

The molecular systematic analyses were carried based on newly determined sequences obtained from the barcode region of the COI marker, and on previously published sequences obtained from the NCBI database. The sequences were aligned on BioEdit 7.0.5.3 software and analyzed with the MEGA 5 software.

Our results suggest the use of the barcode approach as a reliable tool for species identification of Tetraodontidae. Additionally, when used to investigate the specific diversity of the freshwater/estuarine genus *Colomesus*, we were able to identify a new cryptic and endemic

species constantly traded as the South American pufferfish *Colomesus asellus*, a situation which itself requires special attention from the Brazilian border authorities.

Funding Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

Molecular identification of a Buffy-tufted-ear marmoset (Callithrix aurita) incorporated in a group of invasive marmosets in the Serra dos Orgãos National Park, Rio de Janeiro – Brazil

R.S. Carvalho[1,3], D.A. Silva[1,2], S.O. Loiola[1], D.G. Pereira[3], E.F. Carvalho[1,2], H.G. Bergallo[2]

[1] Laboratório de Diagnósticos por DNA, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[2] Departamento de Ecologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[3] Programa de Pós-Graduação em Ecologia e Evolução, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Introduction/Aims: For years illegal pet trade resulted in the introduction of marmosets from the species Callithrix jacchus and C. penicillata in the Atlantic Forest of southeastern Brazil. Among other problems these primates are hardly able to produce identifiable fertile hybrids in crosses among themselves and also with its counterpart, the endangered Buffy-tufted-ear marmoset (C. aurita), which is natural of this region. By sequencing using mitochondrial markers Cyt b, CO I and CO II, this work shows the molecular characterization of a mixed group of marmosets that occur in a range between the National Park's forest and a urbanized area of southeastern Brazil. Methods: The molecular analyses were carried based on newly determined sequences obtained from previously published sequences obtained from the NCBI database. The sequences were aligned on BioEdit 7.0.5.3 software and analyzed with the MEGA 5 software. Results/Discussion: The results showed that the group of marmosets studied is formed by one male individual, whose phenotype and genotype match the endangered species C. aurita, among the others belonging to the invasive species C. penicillata and C. jacchus. This reinforces the importance of studies aimed to elucidating the dynamics of gene flow and the viability of possible hybrids facing the conservation of native species. From the forensics standpoint, the characterization based on three molecular markers ensures a more comprehensive tool for identifying those species combating illegal trade and preserving the endangered C. aurita.

Financial support: CAPES

Finding of DNA barcode from *Papaver* species

B.Y. Seo1, D.-H. Ahn2, E.-A. Cho2, H.H. Oh1,S.H. Lee1, W. Kim3, H.B. Kim4, G.-S. Min2

[1]DNA Forensic Division, Supreme Prosecutors' Office, Seoul, South Korea

[2]Department of Biological Sciences, Inha University, Incheon, South Korea

[3]School of Biological Sciences, Seoul National University, Seoul, South Korea

[4]Life Sciences Research Institute, Biomedic Co., Ltd. Gyeonggi-do,South Korea

Genus Papaver consists of approximately 80 species, which belongs to the family Papaveraceae. Among them, opium poppies such as *P. setigerum* and *P. somniferum* are strongly controlled by law in South Korea and many other countries, while most other species are not. Therefore, discrimination between prohibited species and others is important in the aspect of forensic science. Thirteen universal markers in chloroplasts and an ITS in ribosomal RNA were selected to find most suitable DNA markers which allow differentiation between *Papaver* species. Genomic DNA was extracted from single seeds directly or leaves of young seedling plants. Thirty eight populations out of 16 *papaver* species were analyzed. An opium poppy, *P. somniferum* was well discriminated from all the gene regions used in this study with high inter-specific and low intra-specific. Some other species, especially representatives of section *Rhoeadium* (*P. rhoeas, P. dubium* and *P. commutatum*) or section *Meconella* (*P. nudicaule, P. miyabeanum* and *P. alpinum*) were undistinguishable in many primer sets except *psb*A-*trn*H by low inter-specific variations. The *psb*A-*trn*H sequences, however, showed considerable variations between species and thus was revealed as most promising candidate for barcoding of *Papaver* species.

Molecular genetic assessment of the Glossy Black-Cockatoo, *Calyptorhynchus lathami*, to assist in the investigation of the illegal trade in wildlife.

J.Lee[1], S.Sarre[2], L.Joseph[3], J.Robertson[2]

[1]Northern Territory Police Fire and Emergency Services, Darwin, NT, Australia

[2]University of Canberra, Canberra, ACT, Australia

[3]CSIRO, Canberra, ACT, Australia

The illegal trade in wildlife is a serious and growing crime and one to which Australia is not immune. Worldwide it is thought to cost between US$10 and US$20 billion dollars annually. The trafficking of birds, in particular the parrots, is a known feature of the illegal trade in wildlife. The Glossy Black-Cockatoo, *Calyptorhynchus lathami*, (Psittaciformes: Cacatuidae) was chosen as a model species as it is difficult to breed in captivity and, as the rarest of the Black-Cockatoos, is vulnerable to poaching.

One of the greatest challenges facing the policing of the illegal wildlife trade is the provision of evidence. For DNA evidence to be presented in court, an understanding of the population and genetic structure of target species is required for the results to be interpreted. This study explores a number of DNA technologies that can be utilised firstly to investigate the population genetics of the species as well as to assist in the investigation of instances of illegal trading in wildlife. This includes (i) mtDNA analysis to provide details as to the likely population of origin, and to exclude an individual as coming from a particular matriarchal lineage; and (ii) microsatellite analysis to provide further inference regarding the population of origin, to differentiate among individuals and to investigate pedigrees. In investigating the genetics of *C. lathami*, a great deal of genetic information was obtained that can be used to inform conservation management of the species.

Genetic data of 10 X-STRs in a population sample from Lima, Perú

C. Barletta[1], J.J. Builes[2,3], M.L. Bravo[2], D. Aguirre[2], J. Descailleaux[1], L. Gusmão4

[1] Laboratorio de Genética Humana, Universidad Nacional Mayor de San Marcos, Lima, Perú.

[2] GENES Ltda, Medellín, Colombia.

[3] Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

[4] IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

The Chromosome X-STRs were recently recognized as useful tools in forensic kinship testing, mainly in solving of complex cases. The aim of this study was describe the polymorphism of 10 X-STR loci in a Peruvian population sample, and evaluate their efficiency in forensic practice and paternity testing.

A set of 282 samples, 141 males and 141 females, obtained from unrelated donors of the Lima population were analyzed using 10 X-STRs -DXS6789, DXS9902, DXS7132, GATA31E08, DXS7133, DXS9898, DXS8378, DXS6809, DXS7423 and GATA172D05- through PCR multiplex. The allele frequencies and mutation rates of the ten loci were investigated, and the comparison of allele frequency distribution with other populations (Latin American, Iberian and African) was performed. Statistical analysis was performed using PowerStats and Arlequin software**.**

The female population showed no deviation from Hardy-Weinberg equilibrium at all loci analyzed. DXS7132 was the most polymorphic marker (PIC = 0.7615) with a probability of discrimination in males and females of 0.7913 and 0.9267, respectively. The combined use of these markers produced high values of mean exclusion chance in trios involving daughters and in father/daughter duos of 0.9999752595 and 0.999221451, respectively, demonstrating the usefulness of this set of markers in forensic and kinship analysis. Four mutations were observed in 138 trios father/mother/daughter studied using the same set of X-STRs.

Pair-wise comparisons of allele frequencies distribution showed significant differences for most loci among different populations studied. The study of these X-STRs contributed to the establishment of a database for X chromosome markers in Lima-Perú population.

A multi-gene approach to species identification of forensically important blowflies.

H.Godfrey[1] and J. A.Smith[1]

[1]University of Central Lancashire, School of Forensic & Investigative Sciences, Preston, UK

Introduction and Aims: Many insect species are attracted to cadavers but it is the members of the Blowfly family (Calliphoridae) that are usually the first to arrive. The stage of larvae found on a body can be a useful indicator of time since death, but in order for species specific life cycle data to be applied, accurate species identification is critical. Damaged, unviable or immature specimens can be difficult to identify morphologically and recent work has focussed on the genetic identification of Blowfly species. The aim of this study was to assess the potential of different markers to differentiate UK blowflies and develop a multi-gene assay for species identification.

Materials and Methods: Nuclear (ITS2, 28s rRNA, CAD, Bicoid and Elongation factor 1 alpha) and mitochondrial markers (Cytochrome oxidase I and II, cytochrome b and 16s rRNA), were sequenced from wild-caught specimens of six UK species commonly associated with cadavers (*Calliphora vicina, Calliphora vomitoria, Lucilia sericata, Lucilia illustris, Lucilia caesar and Photophormia terranovae*).

Results and Discussion: Species specific SNPs were identified within most genes enabling the development of a 15-plex SNaPshot assay for identification. Mitochondrial genes exhibited higher levels of inter versus intra species variation with the nuclear markers being more conserved. However problems still exist when identifying closely related species such as *Lucilia illustris* and *Lucilia caesar*.

Resurrecting the Dodo: positive control DNA for species identification

L.M.I. Webster[1], R. McEwing[2]

[1] Science and Advice for Scottish Agriculture, Edinburgh, UK

[2] TRACE Wildlife Forensics Network, Edinburgh, UK

Positive controls are necessary standards for inclusion in forensic tests. When working as expected they demonstrate that methods have been applied correctly, and therefore results can be interpreted with confidence. However, the requirement for positive controls can also introduce problems. For species identification in wildlife DNA forensic testing, it is possible that the DNA sequence of the case sample will be a 100% match to the positive control. While clear results for negative controls will indicate that cross-contamination is unlikely, it would be preferable to have a positive control that will not appear in casework. In addition, for many endangered species, obtaining positive control DNA for species-specific testing can be problematic. Here we present a simple method to use artificially generated positive control DNA from the extinct Dodo.

A Dodo cytochrome b sequence was obtained from genbank and tested for similarity with other sequences. Four hundred bp of the sequence was concatenated with primer sequences from four tests regularly used in the UK for species identification. These were added in such a way that the amplicon length of a case sample would be slightly different from the artificial Dodo control. This construct was then synthesised and validated to determine the windows of applicability for this artifical control in casework.

This artificial control provides a useful, simple and cost-effective solution to the issues of sourcing and using positive control DNA in a forensic context for species identification.

Meat trade: need for international standardization?

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

[1] Forensic DNA Laboratory, University of the Western Cape, Bellville, South Africa

[2] Servicio de Huellas Digitales Geneticas, Universidad de Buenos Aires, Buenos Aires, Argentina.

Extensive substitution of meat products has been detected in the South African market. The evidencing of above 80% substitution of fish products [1] and approximately 70% substitution for red meat products [2,3] produced very different reaction from consumers: whereas the fish substitution remained nearly unnoticed, the red meat substitution gained sensational publicity. This strong reaction likely reflects a South African cultural affinity for red meat, and religious habits such as the prohibition of pork consumption for the Muslims and Jews, and cattle for Indians.

Our results [3] based on cytb and COI DNA sequence information showed a large variety of undeclared wild species, imported undeclared kangaroo and, most concerning, endangered species. Cytb was more informative than COI, and allows for inference of geographic origin on African ungulates. Cytb should be, then, a first choice marker for application to wild meat authenticity.

The complete lack of control of food authenticity in SA resulted in the government initiating a monitoring plan. The consequences of mislabeling and substitution have serious implications for international trade. Recent studies in Europe demonstrated this problem might extend globally, yet international standards for food authentication are not set. Available commercial kits make use of cytb, 12S, 16S or unrevealed DNA sequences for species testing. Most of these would be of limited application in a market with a large variety of related species. The local SA problem would require the generation of a database for genetic information from local ranchers and producers,

and establishment of international standards for meat DNA testing would be required for imported meat.

This work was funded by the National Research Foundation of South Africa (NRF) and the UWC.

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Development of a DNA based molecular technique to authenticate species present in commercial raw meat products.

Y. Dollie[1], M.E. D'Amato[1], P.A. Gouws[1]

[1]University of the Western Cape, Cape Town, Western Cape, South Africa

Over recent years, species identification of animal derived food products for human consumption has gained increasing interest due to numerous public health crises, meat adulteration. Mislabeling is illegal and has raised religious and economic issues. Adulteration detection and identification of adulterants in meat products are crucial for ensuring correct labeling and preventing unfair trade. This is imperative for implementing national standards and to protect consumer preferences.

The aim of this study was to derive a molecular technique to identify the animal species present in a given meat sample, in a simple and cost-effective manner.

Species-specific priers were designed for cow (*Bos taurus*), horse (*Equus caballus*), ostrich (*Struthio camelus*), pork (*Sus scrofa*) and sheep (*Ovis aries*) using the mitochondrial DNA gene (mtDNA) 16S rDNA. A multiplex polymerase chain reaction (PCR) was designed that yields species-specific DNA sized fragments of various lengths, allowing distinction amongst the species. Additionally, cross-reactions with related species were tested. Meat samples from local markets were subjected to polymerase chain reaction (PCR) and the amplified products established the animal species present, due to its respective fragment size.

Overall this study produces a quick and cost friendly method to determine the animal species present in raw meat products and this would indicate if correct food labeling regulations were abide.

A multiplex PCR method to identify bushmeat species in wildlife forensics

N.V. Morf[1], K.L. Wood[2], R. Köppel[3], N. Felderer[3], M. Daniels[3], B. Tenger[2], A. Kratzer[1]

[1]University of Zurich, Institute of Forensic Medicine, Zurich, Switzerland

[2]Tengwood Organization, Wallisellen, Switzerland

[3]Official Food Control Authority of the Canton of Zurich, Zurich, Switzerland

Airports are important hubs for bushmeat trade, that is, the illegal commercialization of wildlife meat, often from endangered species.

While the identification of bushmeat species is sometimes possible morphologically, in most cases genetic characterization would be necessary for precise taxonomic identification. We validated a mtDNA-based species identification method for its application to bushmeat species imported into Switzerland.

This method consists of a multiplex-PCR-setup with 8 primers varying in their specificity to amplify a region of the mitochondrial cytochome b (cytb) gene in different animal classes (mammals, fishes and birds). We tested the method on meat (cooked, dried, smoked or raw) and blood samples from 24 different species, obtained from museums and butchers. Following successful PCR and sequencing, we identified the species of origin using BLAST alignments to the cytb entries in the NCBI nucleotide database. Our method also passed all validation criteria, including repeatability, reproducibility, and robustness.

We then applied this method to identify the taxonomic origin of 250 samples of putative bushmeat confiscated at the airports of Zurich and Geneva between September 2011 and January 2013. For 224 samples, there was sufficient DNA to allow successful sequencing.

The samples belonged to one of four vertebrate groups (mammals, birds, reptiles and fishes): approximately two thirds of the analysed samples originated form wild animals, one third of which are listed in the CITES appendices. Our validated DNA method offers a practical and easy approach for the identification of bushmeat at airports and in general in wildlife forensics.

Wild-Plex: A STR multiplex PCR for the identification of wild European animals in forensic case work

N. von Wurmb-Schwark[1], E. Richert[1], J.-H. Modrow[1], T. Schwark[1]

[1]Institute of Legal Medicine, University Hospital Schleswig-Holstein, Campus Kiel

The classification of biological material that might derive from wild animals can be of great interest, e.g. in insurance cases after a car accident with a possibly involved wild boar or a deer or when suspicious remains are found and the question arises whether they are of human or wild animal origin.

To solve those problems we developed a PCR that amplifies polymorphic short tandem repeats specific for deer, wild boar, wild rabbit, red fox and badger.

Specific primers were chosen from the literature or self-designed using our software program STRAnalyzerv1.5.6. Primers were labeled with 6-FAM and JOE and amplified products were separated and detected on a ABIPrism 3130 using a self-made bin set.

The developed assay allows the simultaneous detection of five different species that can be of forensic interest in Europe. Because of the relatively low detection threshold of about 500 pg and the rather short amplicons the PCR is very suited for forensic case work when dealing with only small amounts of usually also degraded material.

Are these food products fraudulent? Rapid and novel tetraplex-direct PCR assay for meat identification

T. Kitpipit[1], K. Sittichan[1], and P. Thanakiatkrai[1]

[1]Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

**Corresponding author: pthanakiatkrai@gmail.com*

Unintentional contamination and fraudulent labeling of food, especially in halal-certified products, breach both religious and international laws. DNA-based methods are widely employed to identify trace amount of contaminants for law enforcement. Direct PCR has proved successful in the DNA analysis from degraded samples and PCR-inhibited samples, but it has never been applied to meat identification from food products. We aimed to develop a multiplex direct PCR assay for simultaneous identification of four commonly consumed meats without the need to extract DNA. Species-specific primers were designed from the cytochrome *b* gene using the alignment of sequences available on GenBank. The assay was also validated for its specificity, sensitivity, and usefulness in street sample analysis. The results showed that a highly specific and sensitive multiplex direct PCR assay was developed and provided the expected PCR fragment of approximately 100, 119, 133, and 155 bp for pork (*Sus scrofa*), mutton (*Ovis aries*), chicken (*Gallus gallus*), and ostrich (*Struthio camelus*), respectively. Several market samples were tested and a small number of fraudulent labeling was detected. In conclusion, we developed a rapid and inexpensive test for four meat species. The cost- and time-saving assay could be easily adopted in the world food hubs, which are mostly third-world countries.

Funding: The Prince of Songkla University Research Fund (SCI550384S)

Keywords: tetraplex, direct PCR, meat, fraud identification

Human identification through analysis of the salivary microbiome

S.L. Leake[1], G. Greub[2], F. Taroni[1]

[1] Institut de Police Scientifique, University of Lausanne, Lausanne, Vaud, Switzerland

[2]Institut de Microbiologie, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Vaud, Switzerland

Human identification has played a prominent role in forensic science for the past two decades and will continue to do so. The development of techniques to exploit samples, which cannot be satisfactorily analysed at present, is driving the field. High-throughput sequencing techniques have been available for a few years however there are very few examples of their application in forensic science.

This study investigates the potential for bacteria found in the salivary microbiome to be used in a new method for human identification. Three different targets (16S RNA, rpoB *streptococcus* and rpoB gram negative) were chosen to maximise coverage of the salivary microbiome and when combined, they increase the power of identification. Paired-end Illumina high-throughput sequencing was used to analyse the bacterial composition of saliva from two different people at four different time points (t=0 and t=28 days and then one year later at t=0 and t=28 days).

Six major phyla dominate the samples: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria and Spirochetes. *Streptococcus*, a firmicute, is one of the most abundant genera found in saliva and targeting rpoB *streptococcus* has enabled a deeper characterisation of the different *streptococci* species otherwise impossible with 16S alone. We have observed that samples from the same person group together regardless of time of sampling. However, samples taken one month apart group closer together than those taken one year apart. The results indicate that it is possible to distinguish two people using the bacteria found in their saliva.

Genetic canine population data for forensics by 10 STR loci and mitochondrial DNA sequencing in Korea

Seo Hyun Moon[1], Nam Ye Kim[1], Ki Won Park[1], Myung-Haing Cho[2]

[1] Forensic DNA Center, National Foresic Service, Seoul 158-707, Republic of Korea

[2] College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea

Evaluating the population substructure of dogs to estimate the true significance of a STR (Short Tandem Repeat) - based DNA match for use as evidence in forensic science is important. The high frequency of dog hair in crime scenes necessitates the development of mitochondrial analysis as well. The purpose of this study is building STR and mitochondrial DNA database of dogs in Korea. It will be a powerful tool to strengthen a value of dog DNA as evidences in forensic science. Allele frequencies for 10 STRs were analyzed using the StockMarks canine genotyping kit(Applied Biosystems). A pool includes 700 unrelated purebred and crossbred dogs consisting of 20 different breeds. This database will include locus informativeness, allele frequencies, distribution of domestic dog genetic variation, match probability estimates and inbreeding coefficients. Random samples from veterinary clinics in Korea. It is well known that the potential uses of canine mitochondrial DNA as evidence, so it is profiled by sequencing the two hypervariable regions, HV1 and HV2 of the control region. By categorizing canine mtDNA haplotypes, it develop the first public reference database of canine mtDNA in Korea. To effectively use canine microsatellites for forensic identification, it is essential to assess the population substructure in dog populations. Building a population substructure of purebred and crossbred dogs in Korea make us to determine the discrimination of forensic work. Also, mitochondrial analysis is complementary to the canine DNA STR typing.

This work was supported by National Research Foundation of Korea.

Primer template mismatch and its effect of PCR efficiency in relation to species-specific assays

S.S.Tobe[1]

[1]School of Biological Sciences, Flinders University, Adelaide, SA, Australia

Non-human genetic analysis is gaining prominence for forensic purposes. Its uses are however limited due to the difficulties in analysis. Sequencing of a mitochondrial product using universal primers is common in other disciplines, but in forensic science where mixtures are expected it has many downfalls including inconclusive and uninterpretable results. The use of species-specific primers can overcome these difficulties, but these primers are designed based on SNPs within the priming sequence specific to particular species. It is currently unknown how a mutation at these SNP sites will affect the specificity of the primer/template complexes.

Primers specific to dog were developed on the cyt*b* gene and optimised using SYBR Green in RT-PCR to ensure maximum efficiency. Additional primers were developed that contained template mismatches at the 1st, 2nd, 3rd bases (3' end) and combinations of various other locations to simulate template mutation in the priming site. These mismatched primers were reacted under the optimal PCR conditions for the specific primers and any decrease in efficiency was determined using RT-PCR. Further annealing conditions were altered to determine any effect on efficiency of the specific as well as the mismatched primers.

This study demonstrates that a few species-specific SNPs within a priming site are sufficient to provide specific binding even in the presence of similar DNA. It also highlights the danger of using a single species-specific target that could provide false negative results if a mutation event occurs in the 3' end of a priming site. Multiple targets are therefore recommended.

Profiling pythons to combat common illegal wildlife activities

S.A. Ciavaglia[1,2], J.M. Henry[2], A.M.T. Linacre[1]

[1]Flinders University, Adelaide, South Australia, Australia

[2]Forensic Science SA, Adelaide, South Australia, Australia

Reptiles are the most common illegally traded vertebrates leading to many species becoming extinct or highly endangered; yet these species receive relatively low publicity and few genetic tests are available. Sought after as unusual pets and trophies, reptilian skin and meat are also valuable commodities, while certain anatomical features are valued in traditional medicinal cultures. The Australian carpet python is a popular target for illegal trade, breeding and export activities. It provides a model example for which to develop forensic markers that will positively impact wildlife criminal investigation and enforcement both in Australia and worldwide. We report on the initial isolation and characterisation of 24 polymorphic Short Tandem Repeat (STR) loci to enable individualisation and paternity testing of carpet pythons. These novel tetra repeat loci have been examined for their polymorphic content, heterozygosity and species specificity. The loci are being arranged into multiplex reactions exhibiting heterozygote balance with subsequent determination of stutter measurements. Allelic frequency databases of native populations are being developed that will allow not only match probabilities but will potentially predict geographic origin of poached individuals. This profiling system will be subject to rigorous validation for use in legal proceedings.

Funding was provided by the South Australian Department of Justice and the Nature Foundation of South Australia.

Improving mtDNA profiling of dog hair by coding region SNP analysis

S. Verscheure[1,2], S. Desmyter[1], T. Backeljau[2,3]

[1] National Institute of Criminalistics and Criminology, Brussels, Belgium

[2] University of Antwerp, Antwerp, Belgium

[3] Royal Belgian Institute of Natural Sciences, Brussels, Belgium

In casework, mitochondrial DNA (mtDNA) profiling of dog hair is mostly based on the control region. However, the worldwide frequent occurrence of a number of control region haplotypes is detrimental to its application. In this study, variation within the coding region of canine mtDNA was explored to locate single nucleotide polymorphisms (SNPs) that subdivide the haplotypes. The aim is to develop SNP assays that only determine the identity of these discriminative sites.

A method was established to sequence the entire canine mitochondrial genome (mtGenome) according to QA standards. Only two overlapping amplicons are created and 96 sequencing primers enable a double strand sequence coverage. The mtGenome sequence was assembled for 161 dogs selected from a Belgian control region database (Desmyter et al, 2012).

Studying the mtGenome sequences, it was decided to focus the profiling strategy on the three most common canine control region types, each occurring in about 15% of the Belgian population sample. These were subdivided into 53 mtGenome haplotypes. Informative locations were indicated that group them into 24 clusters of mtGenome types with population frequencies of 0,5 to 9%. SNP assays based on these discriminative sites are being developed.

In conclusion, coding region SNP analysis in addition to control region sequencing would benefit the discriminative power of mtDNA analysis of dog hair. Moreover, subdividing only the three most frequent haplotypes could already improve the exclusion probability from 92,5 to 97,1%.

Direct PCR-FINS: non-human species identification without DNA extraction

T. Kitpipit[1], P. Thanakiatkrai[1], and W. Chotigeat[2]

[1]Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

[2] Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

*Corresponding author: thitika.k@psu.ac.th

Forensic DNA analysis is a valuable means for forensic investigation of both human and non-human crimes. Forensically informative nucleotide sequencing (FINS) is one of the several DNA-based techniques used in non-human forensic laboratories worldwide; however, it is costly and time-consuming, which are the most significant obstacles in routine forensic work. Rapid direct PCR – DNA amplification without prior DNA extraction – has been garnering attention lately in the human forensic community and provided high efficiency, but it has never been applied to non-human forensic investigation. Therefore, we aimed to develop and evaluate the possibility and efficiency of using direct PCR-FINS method for species identification from various types of forensically relevant animal samples. Eight biological evidence types (hair, internal organ, muscle tissue, bloodstain, bone, horn, urine, and feces) from three common crime-related animals (tiger, white rhino, and Asian elephant) were analyzed with the three-step direct PCR-FINS method: direct PCR amplification of target DNA with universal primers, sequencing, and phylogenetic analysis using registered sequences on GenBank to confirm the species. All sample types were successfully amplified using direct PCR-FINS method and provided the expected PCR products. Each amplicon sequence and its relevant animal species formed a monophyletic group in a reconstructed phylogenetic tree, meaning that the species of the samples were correctly identified. In conclusion, this newly developed 'direct PCR-FINS method' is rapid, reliable, and meets the requirement of international forensic organizations. It would be beneficial to and provide the highest evidential value possible for non-human forensic DNA community.

Funding: The Prince of Songkla University Research Fund (SCI550385S)

Keywords: direct PCR, FINS, forensic DNA, non-human

Multiplex – direct PCR assay for foodborne pathogen identification: an application in forensic investigation

L. Hasap[1], P. Thanakiatkrai[1], K. Singkhamanan[2], A. Linacre[3] and T. Kitpipit[1,*]

[1]Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

[2]Department of Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

[3]School of Biological Sciences, Flinders University, Adelaide, South Australia, Australia

*Corresponding author: thitika.k@psu.ac.th

Foodborne pathogens present serious concern to human health and can even lead to fatalities. Microbial forensic science thus plays an important role in consumer protection, food security, and even in litigation. The gold standard for pathogen identification, bacterial culture, is costly and time-consuming. A cheaper and quicker alternative will benefit both forensic science and medical diagnosis.

In this study, we developed and validated a molecular-based method called "direct-multiplex PCR assay" to simultaneously detect three common foodborne pathogens – *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Listeria monocytogenes*. Three previously reported species-specific primer pairs were modified and used to directly amplify samples without DNA extraction. The assay was also validated for its specificity, sensitivity, and applied to test several samples obtained from a local market and clinical samples. The results showed the expected PCR fragments of approximately 490, 343, and 209 bp for *Escherichia coli* O157:H7, *Campylobacter jejuni*, and *Listeria monocytogenes*, respectively. The assay was specific to the targeted pathogens and was sufficiently sensitive and robust to effectively analyze market samples and clinical samples. The whole process took less than one hour to complete indicating that the assay is suitable for reliable, rapid, and inexpensive identification of these three foodborne pathogens, which could be useful in microbial forensic investigation.

Funding: Graduate Studies Research Grant, Prince of Songkla University

Keywords: food-borne pathogen, identification, mutiplex, direct PCR

Low-cost direct PCR for wildlife DNA analysis

T. Kitpipit[1,*], P. Thanakiatkrai[1], A. Linacre[2], Y. Lapwong[3], and W. Chotigeat[4]

[1]Forensic Science Program, Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

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

[3] Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Hat Yai, Songkhla, Thailand

[4]Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

**Corresponding author: thitika.k@psu.ac.th*

Direct PCR (PCR amplification with no DNA extraction) has been used successfully in forensic DNA analysis from degraded, process, and inhibited samples using specialized, commercial direct PCR kits. This is attributed to the proprietary chemicals provided in the kits such as pre-PCR buffer and modified DNA polymerases. These reagents can be expensive limiting their widespread adoption in developing countries, where wildlife crimes are often more severe. We report on a novel study to compare DNA polymerases for direct PCR (Phire® Direct PCR, Phire® Hot Start II, Q5®, TopTaq®, and Platinum® *Taq*)and evaluate four chemicals (commercial pre-PCR buffer, phosphate buffered saline (PBS), sodium dodecyl sulfate (SDS), and proteinase K) as a pre-PCR buffer. The study used over 60 samples from six sample types (hair, muscle, bloodstain, bone, horn, and ivory) for each pre-PCR buffer – polymerase combination. The result indicated that PBS, in combination with either the Phire® or Q5® DNA polymerase, showed efficiency comparable to commercially available kits. We observed a rate of more than 95% amplification success and high PCR product concentrations; sufficient for further analysis. The developed direct combination was efficiently used to identify species origin from more than 30-year old museum hair specimens and trace blood sample mixed in vodka. The assay proved to be cost effective and robust; thus, we expect it to be adopted by wildlife forensic laboratories in developing countries.

Funding: The Prince of Songkla University Research Fund (SCI550385S)

Keywords: direct PCR, DNA polymerases, pre-PCR buffers, wildlife forensic

Establishment of an STR multiplex PCR for identification of farm animal species in gastric contents and food

T Schwark[1], V Hessling[1], JH Modrow[1], N von Wurmb-Schwark[1]

[1]Institute of Legal Medicine, University Hospital Schleswig-Holstein, Kiel, Germany

Species identification has become increasingly important in recent years, not only with respect to poaching but also concerning food safety. A common method for species determination is amplification of mitochondrial specific fragments and subsequent RFLP or sequencing analysis. The aim of our study was to establish an STR based multiplex PCR for simultaneous amplification of selected mammals that are commonly used as food source in Europe.

Species specific primers were taken from the literature or designed applying a self-made program (STRAnalyzersoftwarev.1.5.6). DNA was extracted from five different species (horse, cow, pig, wether, goat) and used as template. Fluorescent labeled PCR products were analyzed on an ABI3130 Genetic Analyzer with a self-designed bin set. The assay was then tested on various food samples and also on gastric contents taken during autopsies.

The presented method allows a simultaneous detection of DNA from five different species down to a DNA content of 0.5 ng and can be used also for testing of processed food and gastric contents. It is thus well suited for screening purposes concerning food safety, and also in a forensic context.

Genetic method of species identification of hallucinogenic and other poisonous mushrooms for forensic purposes.

A. Sekuła[1], M. Kowalczyk[1], P. Mleczko[2], Z. Olszowy[3], A.Kujawa[4], T. Kupiec[1]

[1] Institute of Forensic Research, Kraków, Poland

[2] Institute of Botany, Jagiellonian University, Kraków, Poland

[3] Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland

[4] Institute for Agricultural and Forest Environment, Polish Academy of Sciences, Turew, Poland

A common type of case dealt with by forensic laboratories is mushroom poisoning, as a result of suicide attempts, or accidental or deliberate consumption of poisonous or hallucinogenic mushrooms. The classical method of species identification of mushrooms in toxicological analyses is based on morphological, biochemical or physico-chemical analysis of material secured from the patient. Genetic analysis techniques constitute a promising alternative to classical species identification. Scientific studies carried out until now have shown the usefulness of polymorphism analysis of the ITS (internal transcribed spacer) region of nuclear ribosomal DNA (nrDNA) in species identification of fungi.

The objective of the project was the preparation of an ITS1 and ITS2 regions sequence database of the analyzed fungal species and the validation of methods for DNA sequence analysis of these regions in the case of clinical samples. The research material was 200 clinical samples taken from patients admitted to a toxicological clinic with a diagnosis of mushroom poisoning. The dried specimens of over 200 species of fungi collected in different regions of Poland constituted the reference material. Genetic material isolated from samples was amplified with primers specific for the ITS1 and ITS2 regions of nrDNA

and also a primer specific for Basidiomycota. The amplification product was sequenced in both directions and analysed using BioEdit software and then compared with sequences available in the internet data base GeneBank.

The presented method of species identification of fungi together with the created reference data base of sequences of regions ITS1 and ITS2 may be used for forensic or clinical purposes.

Characteristics of the Two Microbial Markers in Vaginal Secretions in Chinese Han Population

F.Song[1], H.B.Luo[1,2], Y.B.Li[1], J.Zhang[1], Y.P.Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

[2]Shanghai Key Laboratory of Forensic Medicine, Institute of Forensic Science, Ministry of Justice, P.R.China, Shanghai 200063, China

Distinguishing vaginal secretions from other forensically relevant biological fluids could significantly aid the forensic investigation, especially in cases of sexual assault. Recently, two microbial markers, *Lactobacillus crispatus* and *Lactobacillus gasseri* for identification of vaginal secretions, were reported. However, the relevant study was not performed in Chinese Han population. In this study, we investigated the characteristics of the two markers in Chinese Han population. We detected 16S–23S rRNA intergenic spacer region by reverse transcription polymerase chain reaction (RT-PCR) assay and characterized the species composition by sequencing 16S rRNA genes. The results revealed that the two microbial markers only expressed in vaginal secretions samples rather than in other body fluids, but they did not express in all female individuals. In conclusion, the inter-individual variation of these two markers should be considered, when the two markers were used for the identification of vaginal secretions in Chinese Han population. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK16B01), the National Natural Science Foundation of China (81273349) and the Science and Technology Committee of Shanghai Municipality (KF1103).

Diatom taxa identification based on single-cell isolation and rDNA sequencing

X.G.Chen[1], J.Zhang[1], Y.Huang[1], Y.P.Hou[1]

[1]Institute of Forensic Medicine, West China School of Basic Science and Forensic Medicine, Sichuan University, Chengdu 610041, Sichuan, China

The identification of diatom taxa can provide important clues for criminal investigation. Conventional morphological identification of diatoms can be inaccurate due to diagnostic characters' variety in life-cycles of diatoms. We developed a single cell rDNA amplification and sequence-based method to diagnose diatom taxon. Single cells of Synedra sp. and Navicula sp. were collected under the microscope. The V4 subregion of 18SrDNA, a good barcode marker for diatoms, was amplified using universal primers (D512 for 18s+ D978 rev 18s). The PCR mixture was heated to 95°C for 10 min before the routine amplification to allow the diatom to burst and liberate their DNA. The PCR products were purified, quantified and commercially sequenced. Uncorrected p-distances were computed using MEGA5.0 with 95 reference sequences downloaded from Genbank. We obtained sequences smaller than 500bp from each strain using individual diatom cells as the source of template. Uncorrected p-distances analysis identified the diatom's genera but failed to diagnose with species. This could be the result of the limitation of the reference data and the variety of barcode marker. This method could be used as supplement of the conventional morphological diatom test for forensic application. This work was supported by the Five-twelfth' National Science and Technology Support Program of China (2012BAK16B01), the National Natural Science Foundation of China (81273349).

Theme 14: Forensic medical matters

Heroin Addictions in Italians: Evaluation of OPRM1 Genetic Variants by Case-Control Association Study

L. Buscemi[1], C. Turchi[1], N[1]. Onofri, F. Ramberti[1], A.Tagliabracci[1]

[1]Institute of Legal Medicine, Ancona, Italy

Drug addiction is a chronically relapsing disorder characterized by compulsion to take the drug and lost of control in limiting intake. The opportunities to analyse the genetic variations related to the risk of addiction are of interest to forensics, who beside their involvement in drugs-related fatalities may also be required to assess driving and working ability as well as permanent invalidity due to drugs-related conditions.

Several genetic variants have been shown to be associated with heroin addiction. The most investigated gene is OPMR1 that encode the μ-opioid receptor.

The purpose of this study was to examine the contribution of genetics variants in OPRM1 to the susceptibility to addiction.

15 SNPs in and around the OPRM1 gene were investigated in 101 Italians with heroin addiction and in 106 control individuals. Case subjects were recruited at the Institute of Legal Medicine of Ancona (Italy) among individuals who died from acute opioid intoxication and among subjects with history of heroin dependence during the procedure for the reinstatement of their driver's license.

Case-control comparisons for allele and genotype frequencies showed new variants of OPRM1 associated to susceptibility of

heroin dependence. The allele frequencies of 4 markers were significantly different between heroin-dependent subjects and healty controls: rs1294091 (P=0.0065), rs1323042 (P=0.0284), rs2075572 (P=0.0351), rs3798683 (*P*=0.0057). Comparison of genotype frequencies showed significant differences for 2 polymorphisms: rs1294091 (P=0.0123) and rs3798683 (P=0.0284).

The identification of genes predisposing individuals to addiction could enhance prevention measures.

Influence of *CYP2D6* and *CYP2C19* Genotypes on Venlafaxine metabolic Ratios and stereoselective Metabolism in Forensic Autopsy Cases

L. Karlsson[1], H. Gréen[1,2], A.L .Zackrisson[2], B. Carlsson[3], M. Josefsson[2] , F.C. Kugelberg[1,2]

[1]Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden.

[2]National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden.

[3]Clinical Pharmacology, Linköping University Hospital, Linköping, Sweden.

Introduction/aims: Venlafaxine (VEN), a racemic antidepressant, is mainly metabolized by CYP2D6 to its active metabolite O-desmethylvenlafaxine (ODV) and a minor metabolite N-desmethylvenlafaxine (NDV) is formed by CYP3A4 and CYP2C19. In the present study we investigate whether polymorphisms in the *CYP2D6* and *CYP2C19* genes influence the metabolic ratios and enantiomeric S/R-ratios of VEN and its metabolites in blood from forensic autopsy cases.

Methods: The study material included 116 postmortem cases found positive for venlafaxine during toxicological screening. VEN and its main metabolites ODV, NDV and N,O-didesmethylvenlafaxine (DDV) were determined with an enantioselective LCMSMS method. *CYP2D6* (**3-*6* and gene-duplications) and *CYP2C19* (**2-*4,*17*) genotypes was determined by Pyrosequencing. The influences of the genotypes on the metabolic- and S/R-ratios were determined using multivariate ANOVA.

Results: The median concentrations of VEN and ODV in all cases were 0.6 and 0.5µg/g, respectively. The *CYP2D6* genotype was shown to significantly influence the concentration ratios of ODV/VEN (P=0.009), DDV/NDV (P=0.0076) and DDV/ODV (P=0.05). The NDV/VEN (P=0.05), DDV/ODV (P=0.0041) and DDV/VEN (P=0.003) ratios were significantly influenced by *CYP2C19* genotype. The S/R-ratios of VEN were significantly influenced by both *CYP2D6* and *CYP2C19* genotype (P=0.0092 and P=0.0054, respectively). CYP2D6 poor metabolisers had lower S/R VEN ratios and CYP2C19 poor metabolisers had high S/R-ratios of VEN in comparison.

Conclusions: Our results show that the *CYP2D6* genotype influences the O-demethylation (VEN→ODV and NDV→DDV) whereas *CYP2C19* influences the N-demethylation (VEN→NDV and ODV→DDV) of VEN and its metabolites. In addition, we show a stereoselective metabolism where CYP2D6 favours the R-enantiomer whereas CYP2C19 favours the S-enantiomer.

Legal aspects and forensic value of the forensic study of health-related information

I.O. Perepechina[1]

[1] Lomonosov Moscow State University, Moscow, Russian Federation

When there is no a suspect, the possibility of gaining of any search information on the individual who has left traces is critical. Great achievements in the area of medical molecular genetics resulted from the realization of the Human Genome Project can potentially be a solid basis for forensic research. However, study of health-related regions is considered to be vulnerable as fraught with potential risk for civil rights of the individual.

The article deals in detail with both national and international legislations in applicable to the admissibility of forensic research health-related information. It also studies the potential and prospects of such a research for forensics, given its complexity and reliability.

The consideration shows that in spite of a number of problems, research of health-related DNA regions is of forensic value and it is able to add new dimensions for police in investigating crimes. The analysis of the worldwide legislation suggests that such an examination conducted with search aims, without inclusion of information into the DNA database, except for a few legislations might be carried out within the existing legal framework. Discussion of the ethical aspects of the admissibility of such examinations should take into account that the impunity of criminals, in turn, violates the right of the victims, their relatives and the society to recompense the offenders, as well as the right of the perpetrators' future victims for life. Such research, if done, requires very strict detailed legal regulation. The problem in question may concern also other novel approaches.

Mutational analysis of TTN, TCAP and TPM1 in cardiomyopathy

K. Maeda[1], C. Murakami[1], W. Irie[1], M. Oishi[1], C. Sasaki[1], N. Nakamaru[1], S. Nakamura[1], K. Kurihara[1]

[1]Department of Legal Medicine, Kitasato University School of Medicine, Sagamihara, Japan

Cardiomyopathy is a primary myocardial disease that causes of sudden death. A number of mutations in genes of Z-disc proteins of the cardiac sarcomere have been found to be responsible for various cardiac dysfunctions in cardiomyopathy. In this study, comprehensive mutational screening of 3 sarcomeric genes was performed to determine the frequencies and types of mutations in sudden death cases caused by cardiomyopathies.

DNA was extracted from blood specimens of 14 cases of hypertrophic cardiomyopathy (HCM), 18 cases of dilated cardiomyopathy (DCM) and 3 cases of Arrhythmogenic right ventricular cardiomyopathy (ARVCM) with informed consent of their family members. Primers were designed to amplify exons of the following genes: N2A region of TTN (titin), TCAP (telethonin) and TPM1 (alpha-tropomyosin). The resulting sequence data were compared with the reference sequence available on the NCBI database.

One nonsynonmous mutation (V9710I) and synonymous mutation (I9726=) in TTN were identified in one DCM case. Moreover, I9726= was also found in one HCM case and Q9705= was found as a new mutation in another HCM case. One polymorphic synonymous mutation was detected in each TCAP and TPM1. V9710I found in TTN was located on binding region to cardiac ankyrin repeat domain, which increases the expression in heart failure. These results suggested that the mutation changed the function of the protein and caused the cardiomyopathy.

It is necessary to analyze the other sarcomeric genes and clarify the relationship with etiology.

HLA typing from sequence data originated by the advanced technology

M. Osawa[1], Y. Inaoka[1], S. Suzuki[2], Y. Ozaki[2], E. Ochiai[1], K. Miyashita[1], T. Shiina[2]

[1]Department of Forensic Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan

[2]Department of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan

The advance of sequencing technology enables us to obtain a huge scale of DNA sequence data. The complete sequencing of the HLA region on chromosome 6, comprising of 3.4 Mb, is appropriate for such advanced application. In the present study, we determined the five loci of *HLA-A, -B, -C, -DRB1, and -DQB1* of class I and II regions in a single chip that worked on the Ion Torrent PGM (Life Technologies). DNA specimens were analyzed after emulsion PCR using the barcoded-library DNAs. The amplicons were loaded onto the Ion 316 chip, and subsequently sequenced using 125 sequencing cycles according to the Ion Sequencing 200 kit user guide. Reference sequences used for mapping of the sequence reads were selected by nucleotide similarity searches with HLA allele sequences in the IMGT-HLA database using the BLAST program (http://genome.ucsc.edu/). The average of reading length was approximately 130 bp, and each HLA locus was typed at 6 or 8 digit levels in all specimens. The highly divergent loci having less than 0.11 of allele frequencies should be helpful for the forensic purposes like personal identification. Moreover, in the analysis to 15 pairs of relatives, including parent-offspring, full siblings, and second and third degrees, the shared alleles were identified as well as the occurrence like recombination events, which will be helpful to construct characteristic HLA haplotypes.

Specific detection of Japanese aconite using multiplex PCR

N Nakayashiki[1], M Fujita[1], K Dewa[1]

[1]Department of Legal Medicine, Iwate Medical University School of Medicine, Shiwa-gun Yahaba-cho, Japan

Aconite species (subgenus *Aconitum*) are one of the most dangerously toxic plants in Japan. Cases of aconite poisoning caused by unintentional eating of the plant are not rare in Japan, and aconitine, the primary toxic ingredient of aconite, has been also used to commit suicide and murder. In general, the presence of aconite poisoning is confirmed based on whether aconitine or related alkaloids are detected in the blood or body fluids of the victim; however, such evidence related to aconite itself has not been investigated in detail. The aim of this study was to identify the presence of Japanese aconite using molecular biological methods.

More than 40 aconite samples gathered from the northern area of Japan and evidences obtained from four poisoning cases were investigated. Four aconite loci composed of the ITS region in nuclear DNA and three regions in chloroplast DNA (matK, trnL-trnF and psbA-trnH) were used to identify the presence of aconite DNA. Tetraplex PCR followed by electrophoresis was employed and the amplicons were sequenced.

Four PCR products ranging from 128 to 252 bp in size were clearly detected in all of the aconite specimens, and the subsequent sequence analysis revealed many nucleotide substitutions and insertion/deletion differences in the aconite samples. This method should be a specific and sensitive technique for identifying Japanese aconite itself and such sequencing information would be useful to specify the individual types of aconite.

Can genomic analysis be the answer to autologous blood transfusion detection?

N. Frascione[1], D.A. Cowan[1], I. Rebollo-Mesa[1], M. Arno[1], B. Daniel[1]

[1]King's College London, London, United Kingdom

Although autologous blood doping is prohibited by the World Anti-Doping Agency, no method for its detection has been devised. The practice entails the removal of several units of blood from the athlete's own body. Red cells (RBCs) are isolated and stored for several weeks then re-introduced into the athlete prior to competition. Several proteomic and biochemical changes occurring to RBCs during storage have been identified but these are quickly reversed when the blood is re-introduced and physiological norms are returned. Genomic analysis has been limited by the long-held belief that mature erythrocytes lack RNA. Only very recently, studies have provided evidence of microRNA (miRNA) transcripts in RBCs. The aim of this study was to identify storage susceptible miRNAs that could be used as markers for autologous transfusion detection.

Blood was collected from healthy volunteers and processed to obtain packed RBCs. Microarrays were used to quantify miRNA levels in fresh and stored samples. Statistical analysis was carried out to identify transcripts that change significantly during storage. Further validation of the identified transcripts was achieved through real-time PCR.

Our data revealed statistically significant transcriptomic changes when fresh and stored RBC preparations were compared, indicating that storage affected a well-defined subgroup of miRNA transcripts. By combining some of the selected markers, a good prediction in distinguishing fresh and stored red cells was obtained. These results could provide the basis for the development of a test for autologous blood doping detection in which 'doped blood' can be identified because of abnormal RBCs' RNA patterns.

Cardiac Arrhythmias and Epilepsy: Genetic testing in 42 cases

Partemi S. [1-2], Coll Vidal M. [2], Oliva A. [1], Campuzano O. [2], Allegue C. [2], Pascali VL MD[1], PhD, Tassinari CA MD[4], Zara F PhD[5] ,Striano P.[5], Brugada R[2].

[1] Forensic Medicine Institute, Catholic University, Largo Francesco Vito 1, 00135, Rome, Italy

[2] Cardiovascular Genetics Center, University of Gerona, Spain

[4] Department of Neurological Sciences, University of Bologna, Bologna, Italy

[5] Department of Neurosciences "G. Gaslini" Institute University of Genoa, Genoa, Italy

Introduction and aims Epilepsy affects approximately 1% of the US population and sudden unexpected death in epilepsy is a significant cause of death for this people and it accounts for up to 17% of all cases of death, which increases the rate of sudden death by 24-fold as compared to the general population. The underlying mechanisms are still not elucidated, but recent researches suggest the possibility that a common genetic channelopathy might contribute to both epilepsy and cardiac disease to increase the incidence of Sudden unexpected death in Epilepsy (SUDEP) The aim of this study was to characterize a large cohort of cases with epilepsy and heart rhythm disorders and to identify variants in genes coding for ion channels co-expressed in brain and heart that could play a role in some deaths.

Materials and methods Patient genomic DNA was extracted from blood and analyzed for mutations: genetic testing was performed of the 3 major (KCNQ1, KCNH2 and SCN5A) and 3 minor (KCNE1, KCNE2 and KCNE3) LQTs susceptibility genes and of the genes encoding for the Beta subunits of Sodium Channel (SCN1B, SCN2B, SCN3B and SCN4B).

Results and discussion Genetic testing revealed 10 nonsynonymous variants, 3 of them previously never reported according to international databases: mutations in the SCN5A (40%) were most prevalent followed by KCNQ1(30%), KCNH2 (20%) and KCNE2 (10%). Only a patient carried multiple mutations (SCN5A and KCNH2). Only one mutation was nonsense whereas the remaining 9 were missense.

Finally, putative pathogenic disease-causing mutations in ion channel genes were detected in 24% of unrelated patients with epilepsy.

KCNH2 mutation in a family with long QT syndrome, epilepsy, and SUDEP

Partemi S [1], Pezzella M[2], Paravidino R[2], Pascali VL [1], Tassinari CA[4], Striano S [5], Zara F[2], Oliva A[1], Brugada R[3], Striano P[2]

[1] *Institute of Legal Medicine, Catholic University, School of Medicine, Rome, Italy*

[2] *Pediatric Neurology and Neuromuscular Diseases Unit, Department of Neurosciences; Institute G. Gaslini, University of Genova, Genova*

[3] *Cardiovascular Genetics Center, Institut d'Investigació Biomèdica Girona-IdIBGi and Universitat de Girona, Girona, Spain*

[4] *Department of Neurological Sciences, University of Bologna, Bologna, Italy*

[5] *Epilepsy Center, Federico II University, Napoli, Italy.*

Introduction and aims Sudden unexpected death in epilepsy is a catastrophic complication of epilepsy, causing up to 18% of patient deaths. A leading hypothesis suggests a pathogenic hyperexcitability that could underlie both epilepsy and cardiac arrhythmias, leading to death. We report a kindred featuring the LQTS, idiopathic epilepsy, and increased risk of sudden death.

Materials and methods Two 18 year-old dizygotic twin sisters were referred to Epilepsy Center for myoclonic jerks at upper limbs and occasional tonic-clonic seizures started from age 13 and 15 years. Interictal EEG showed normal background activity and generalized spike-wave and spike-slow wave complexes, increased by sleep. Surface electrocardiography revealed marked QT prolongation and abnormal T-wave. Family history revealed that their mother and grandmother suffered from long QT syndrome and they died suddenly. One of the twin sisters, at age 18 years, was found dead lying prone her bed at morning.

Results and discussion This family showed the association of idiopathic epilepsy, LQTS, and sudden death. LQTS may be easily misdiagnosed with epilepsy and treated with anticonvulsants. Direct sequence analysis of genomic DNA revealed in both sisters a novel non-synonymous heterozygous missense mutation in exon 2 of the KCNH2 gene. KCNH2, responsible for LQT2, is a ether-a-go-go-related gene which encodes the α-subunit proteins of voltage-gated K+ channel, expressed in several tissues such as heart and brain. Our observation confirms the possibility that KCNH2 mutations may confer susceptibility for recurrent seizure activity, supporting the emerging concept of a genetically determined cardiocerebral channelopathy. Nevertheless, this observation further corroborates a pathogenic link between idiopathic epilepsy and LQTS.

Theme 15: Validation studies, standards and quality control issues

The Ge.F.I. DNA Proficiency Test: year-one experience.

A. Tagliabracci[1], V. Onofri[1], C. Previderè[2], P. M. Schneider[3], F. De Stefano[4]

[1] *Dipartimento di Scienze Biomediche e Sanità Pubblica, Sezione di Medicina Legale, Università Politecnica delle Marche, Ancona, Italy*

[2] *Dipartimento di Sanità Pubblica, Neuroscienze, Medicina Sperimentale e Forense, Sezione di Medicina Legale e Scienze Forensi, Università di Pavia, Italy*

[3] *Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany*

[4] *Dipartimento di Scienze della Salute, Sezione di Medicina Legale, Università di Genova,Italy*

The experience of many years of collaborative exercises of the Ge.F.I. (the Italian Speaking Working Group of ISFG) together with the existing GEDNAP and GHEP-ISFG proficiency testing programs showed clear evidence that the reduction of errors is closely related to increased standardization, as well as to external quality controls, not only of methods and technologies used but also of the interpretation of results.

On this basis and in agreement with ISO/IEC 17025 standards and recommendations of the ISFG Paternity Testing Commission, the Ge.F.I. has launched the DNA Proficiency Test in 2012 consisting of a representative example of casework that laboratories routinely analyze. This allows to simulate situations of major interest for the scientific community (i.e. mixed stains and LT-DNA) to assess the interlaboratory variability, the extent and the type of errors observed in the participating laboratories, in order to implement guidelines and recommendations to overcome potential quality problems.

A total of 26 Police, University and private laboratories belonging to Italy, Switzerland and Germany submitted results on two reference samples and two mixed forensic stains. Data included characterization of the nature of biological fluids, up to 24 autosomal STRs, Y-STRs, mtDNA, biostatistical calculation and theoretical kinship investigation. The participation for autosomal and Y typing was massive; in addition, about 38% of the laboratories submitted mtDNA data. Interestingly, 77% of the laboratories participated in genotyping frequency/likelihood ratio test as well as 65% in theoretical kinship exercise, demonstrating the great interest in statistical interpretation of forensic casework.

Training of legal professionals in DNA evidence

Annelize van der Merwe[1], Arnold Greyling[2,3], Antonel Olckers[1,4]

[1] *DNAbiotec (Pty) Ltd, Pretoria, South Africa*

[2] *ForPsych Consultants (Pty) Ltd, Perth, W.A., Australia*

[3] *Forensic DNA Consultants (Pty) Ltd, Pretoria, South Africa*

[4] *Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa*

The South African Criminal Legal System is based on Roman Dutch law. Court proceedings are led by a single presiding officer of the court. Prosecutors and defence advocates present the court with evidence in an adversarial manner. This system has inherent advantages and disadvantages and therefore the training of legal professionals in

handling DNA evidence in court is important. The prosecutors resort under the National Prosecuting Authority and the defence advocates act independently or under the auspices of Legal Aid South Africa.

Education curricula of legal professional do not include forensic science evidence. Principles such as evidential value in the forensic context are not addressed. Training of legal professionals with our Essential DNA Evidence™ Course has been a multiplier of forensic science knowledge in the legal profession in South Africa. We present prosecution and defence perspectives in an unbiased manner, compensating for the possible subjective interpretations of evidence that may be presented in court. Forensic evidence is subsequently carefully evaluated prior to being court presentation thus improving court efficiency, and allowing for a more focussed approach to the presentation of evidence. Approaches to the customisation of course content that adds value has been identified via evaluation of training programmes.

Experience has shown that legal professionals have the ability to incorporate relatively complex scientific concepts into their legal arguments if provided with the appropriate training opportunity. Appropriate training in DNA evidence has made the court process more effective, both in terms of time and costs, and ultimately serves justice.

DNA evidence in South Africa: lessons learned

Antonel Olckers[1,2]

[1]DNAbiotec (Pty) Ltd, Pretoria, South Africa;

[2]Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

A trend has been noticed over the past 15 years in the South African courts. This trend has a multi-factorial origin and highlights the problems faced in the use of forensic science evidence in court.

Although there have been improvements on how DNA evidence is gathered and presented in court, due to the fact that certain cases have been contested, multiple issues remain that have not yet been addressed. These issues include: accreditation, regulation of the forensic science profession, continued education, training of court officials, quality assurance, biased testimony, lack of transparency with regard to processes and procedures followed in the forensic community, incorrect interpretation of DNA evidence, lack of scientific knowledge by forensic scientists, awareness in the legal profession and an over emphasis on prosecuting perspective. These same aspects continue to plague current cases.

The role of the media and circumstances related to high profile or high priority cases influence the manner in which forensic evidence is handled, from collection to presentation in court. Superimposed on this is the factor of the financial means of the defendant. All these factors align to bring about justice or not. Justice is thus not yet a given to all victims of crime in South Africa, as the Constitution mandates.

Despite the above, the window of opportunity to address the above has not yet passed. However, it will take continuous and concerted efforts from the scientific and legal professions to bring about the appropriate change to facilitate justice for all in South Africa.

Forensic Science in South Africa: Status of the Profession

Antonel Olckers[1,2], **Ryan Blumenthal**[3], **Arnold Greyling**[4,5]

[1]DNAbiotec (Pty) Ltd, Pretoria, South Africa;

[2]Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa

[3]Dept of Forensic Pathology, School of Medicine, Faculty of Health Sciences, University of Pretoria;

[4]ForPsych Consultants (Pty) Ltd, Perth, W.A., Australia;

[5]Forensic DNA Consultants (Pty) Ltd, Pretoria, South Africa

The forensic science profession in South Africa is currently unregulated. There is also no national body that allows for the continued development or professional interaction of forensic scientists.

The above has brought about a dire need to regulate the profession, in order to:

- To provide the victims of crime, the criminal justice system and the crime investigation infrastructure in South Africa with an independent body of forensic science expertise
- To provide the public and government with access to an informed and unbiased entity that can provide guidance and assistance on matters of forensic science
- To provide for an ethical framework for practitioners of forensic science, thereby ensuring transparency of forensic science practices in South Africa that will allow for the building of trust between all role players
- To assist the criminal justice system through access to a register of forensic scientists
- To prevent dominance in terms of numbers of forensic practitioners associated with a single body
- To assist in the development, establishment, registration and growth of forensic science as a profession

To attain the above objectives it is envisaged that the founding of a Forensic Science Society in South Africa is of critical importance. To this end we will discuss several avenues that are being followed at present in order to provide structure to the profession of forensic science in South Africa.

Forensic DNA Laboratory Automation – Principles and Guidelines

A.J. Greyling [1,2]

[1]ForPsych Consultants (Pty) Ltd, Perth, WA, Australia

[2]Forensic DNA Consultants (Pty) Ltd, Pretoria, South Africa

Automation of forensic DNA laboratory processes by means of robotics, commercial equipment modules, process integration and software applications is changing the landscape of forensic DNA typing laboratories significantly.

There is a lack of clear guidelines for project managers, laboratory managers and forensic scientists on strategies for the operational implementation of new technologies. This is reflected in the failure rate of projects in the forensic DNA testing environment. Forensic laboratories are liberally scattered with the remains of failed projects to automate and integrate processes.

We will present a set of guidelines and strategies based on experiences in forensic laboratory automation. The primary example used to discuss this set of guidelines - is an operationally proven, fully automated forensic DNA laboratory using robotics, liquid handlers and layers of custom developed software solutions interfacing with a LIMS to integrate the processes of DNA extraction, quantification, PCR, capillary electrophoresis and automated data analysis. It requires no human intervention post submission of evidence, apart from reagent re-stocking.

Frequently, the primary motivations for laboratory automation consist of reducing human error, improving reliability of testing, increasing production capacity and to reduce costs in order to meet increasing demands for fast and reliable DNA testing. The fallacies in some of these perceptions will be discussed. The presence of a forensic laboratory technology strategy and use of proper technology management principles are shown to be the ultimate determining factors in the success or failure of forensic DNA laboratory process automation.

Successful validation of a fully automated sample lysis workstation

B.J. de Jong[1], M. Antonie[1], A.J. Hardon[1], D. van den Bergen[1] and A.L.J. Kneppers[1]

[1]Netherlands Forensic Institute (NFI), The Hague, The Netherlands

Sample lysis and DNA extraction can be regarded as quantity and quality bottlenecks in the DNA profiling process, the latter influencing the amount, quality and traceability of forensic samples. This explains why the majority of forensic laboratories choose manual lysis and extraction methods when processing crime scene samples. However, manual processing has an increased risk of user-errors, e.g. sample-misplacements and (cross) contamination, potentially rendering critical samples useless.

The NFI recently validated a fully automated system for sample lysis - the AutoLys workstation, developed in collaboration with Hamilton Robotics. We have carried out the developmental validation of the AutoLys system and concluded it offers a solution to drastically reduce user-errors, increase DNA yields, gain in productivity, while maintaining quality. The validation of the DNA purification (available on the same system) is ongoing.

This presentation outlines the validation study setup and results that include: 1) initial testing of prototypes, 2) protocol optimization, and 3) testing of simulated crime scene samples. The AutoLys system successfully processed all commonly found forensic sample types, producing high quality extracts, containing on average 12.1% more DNA than manually extracted samples (determined by qPCR and STR-profiling). The optimized robotic protocol minimizes the risk on cross-contamination (no cross-contamination detected in over 500 samples tested) and retains the flexibility to adjust chemically relevant variables specific to a laboratory's individual requirements. The fully automated process enables complete sample-tracking, drastically reducing the risk on user-errors. The fully automated system frees up resources and will help a laboratory to significantly reduce backlogs.

Integrated Forensic DNA Data Management and Anaylsis- A scalable enterprise solution for forensic DNA laboratories

A T. Minn[1], B. Kays[1], J. Deng[1], G. Lam[1], S. Vijaychander[1], M. Karpagavinayagam[1], C. Dallet[1], J. Benfield[1], J. Schroeder[1]

[1]Human Identification, Life Technologies, Foster City, CA 94404, USA

Forensic DNA laboratory workflow management and data analysis are daunting tasks that share a common requirement: efficient extraction of useful information from related--but disjointed—data sets, stored across a variety of laboratory instruments and systems. Furthermore, as the scale and size of a laboratory increases, so, too, does the complexity and sheer amount of data requiring storage, management and analysis. Life Technologies has developed a new enterprise software solution suite that seamlessly integrates volumes of raw data, processing information, analyzed profiles and analysis reports that are generated throughout the DNA processing workflow during sample accessioning, extraction, quantification, amplification to CE, fragment and profile analysis. The software solution is highly configurable to fit specific laboratory workflows, standard operating procedures, chemistries, as well as, analytical and reporting requirements. The system allows for automated data transfer and integration with various forensic DNA laboratory instrumentation and systems including Life Technologies' real-time PCR and Capillary Electrophoresis instrumentation, as well as, its Genemapper™ ID-X analysis software. The software supports the generation of scalable local DNA profile databases capable of holding millions of profiles that can be searched using a standard identity by state method, as well as, a novel familial search algorithm developed by Life Technologies. Additionally, the system enables user interface and output report configuration, as well as, includes many features to insure data security and integrity. The characteristics, configurability, and analytical search capabilities of the software will be presented.

For Forensic and Paternity Use only.

Additional sequence characterization of NIST SRM 2391c: PCR-Based DNA Profiling Standard

C.R. Hill[1], M.C. Kline[1], M.D. Coble[1], P.M. Vallone[1]

[1]National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, USA

The NIST Standard Reference Material (SRM) 2391c: PCR-Based DNA Profiling Standard was designed for use in the standardization of forensic and paternity quality assurance procedures for fragment-based typing short tandem repeat (STR) alleles generated by the polymerase chain reaction (PCR). The SRM is intended for law enforcement laboratories, non-clinical research purposes, and for assigning values to in-house control materials. Certified genotypes of the 6 components A-F were assigned for 24 autosomal and 17 Y-STR markers plus Amelogenin using concordance testing between commercial kits. Selected Sanger sequencing characterization was performed for the alleles of 11 STR markers when only one PCR primer set was available for fragment-based typing.

Our goal is to characterize the STR loci in components A-C by Sanger sequencing methods for the STR repeat regions and adjacent flanking regions. Core U.S. and European STR autosomal loci will be characterized first followed by Y-STRs present in commercial kits, and non-core loci. Additional characterization of the SRM is intended to support the emerging interest in next-generation sequencing technologies for forensic typing applications. Sanger methods will characterize underlying polymorphisms (sequence, insertion-deletion, variation in complex motifs) typically not detected by fragment-based typing. The sequenced regions will include the commercial or known PCR binding sites commonly implemented in fragment-based typing.

Joining the ranks: South Africa on track to adopting DNA legislation

D.N. Swanepoel[1,2]

[1]Forensic DNA Consultants (Pty) Ltd, Pretoria, South Africa

[2]The DNA Project, Cape Town, South Africa

South Africa has recently been at the epicentre of a spate of horrific violence, with reports of rape and murder making headlines almost daily. Disturbingly regarded as the 'rape capital of the world', South Africa has never before required a more urgent need for the effective use of DNA profiling in aiding investigations as now. Although conducting DNA profiling since 1998, South Africa has had no supporting legislative framework thus far *.

The *DNA Project*, a non-profit organisation, has been actively lobbying to secure such legislation, as well as provide DNA awareness training to a variety of first on crime scene individuals and organisations. As with all new DNA legislation, there arises an essential need to intensify training and awareness around the DNA process in order for DNA evidence to hold the evidential value that it should. Formal and functional training in all aspects of the DNA process will be a requirement once legislation is enacted.

We reflect on key events that have brought us to the point we are at today and look to where we should move from here. Enacting legislation is only the first step on a journey to effectively utilise DNA profiling resources and it is necessary that significant investment be continually made towards the improvement and advancement of this exceptional technology and tool.

* DNA legislation is currently under review and, pending the outcomes of the review process, significant developments may have been made at the time of presentation.

A forensic laboratory information management system for daily case work

R. Nixdorf[1] ,F. Götz[2]

[1] Kriminalwissenschaftliches und -technisches Institut, Landeskriminalamt Sachsen, Dresden, Germany

[2] Qualitype GmbH, Dresden, Germany

The number of forensic samples is rapidly growing nowadays while it is necessary to speed up their analysis by concurrent increase of quality. A way to meet this challenge is the automation of lab processes in combination with a laboratory information management system (LIMS).

Our Abetter LIMS supports the whole process of forensic case work from registration of forensic materials and samples, analysis of samples by pre tests, PCR and fragment analysis, up to the automatic creation of final reports.

Sample analysis and interpretation is simplified and expedited by the integration of fsa and hid raw data analysis of sequencer data as well as comprehensive biostatistical calculations including a reference database with allele frequencies, test kits and markers.

Standard operating procedures (SOPs) can be easily implemented by definition of specific testing protocols for sample analysis and data collection while supporting lab devices and robots. All critical steps can be monitored by the use of the integrated quality assurance systems such as contamination controls and audit trail.

Furthermore, you can manage maintenance intervals and repair events of devices as well as track lots, expiry dates and approvals of reagents according to ISO 17025.

Thus, a LIMS can shorten the turnaround time of samples in laboratories and improve the quality assurance by supporting all necessary guidelines for forensic labs.

From Sample Collection to Report Generation- a New Integrated Workflow Solution for Paternity and Relationship Testing

J. G. Shewale[1], D. Wang[1], C. Zhong[1], S. Gopinath[1], J. Mulero[1], J. Deng[1], P. McClurg[1], C. Dallett[1], A. Minn[1]

[1]Life Technologies, Foster City, California, USA

Short Tandem Repeat (STR) profiling has become the global standard for simple paternity and complex relationship testing. Sample profiling efficiency has been significantly improved in the past decade since biological samples collected for relationship testing are from a single source donor and typically contains ample high quality DNA. Nevertheless, there is an acute need to improve the efficiency of upstream and downstream steps in the relationship testing workflow to achieve greater laboratory efficiency and reduce total program costs. Major bottlenecks noted by many relationship testing laboratories include performing paternity index calculations, generating reports, tracking sample information and searching for information from different systems in the lab. We have developed a new integrated workflow solution for relationship testing comprising improvements in sample collection, chemistry and software. These include a range of efficient sample collection devices, such as 4N6FLOQSwab™ and NUCLEIC-CARD™, Identifiler® Direct and VeriFiler™ Direct STR amplification kits, GeneMapper® ID-X expert system and our recently developed *Paternity System* for complete data storage, access, analysis and reporting. Probability of paternity value for the 21 STR loci is 2.09E-25, 1.51E-26, and 2.61E-25 for Caucasian, African American, and Hispanic populations, respectively. Our novel *Paternity System* is heavily configurable to fit the specific requirements of the laboratory enabling seamless integration of the instrumentation and software. In addition, the solution includes a relationship analysis and reporting tool that can be used for simple paternity, complex relationship analysis and report generation. Principles and advantages of these advancements over traditional workflow will be presented.

Colombian results of the interlaboratory Quality Control Exercise 2012

J.J. Builes[1,2], J.F. Castro[1,2], C.M. Velilla[1], A Manrique[1,2], D.P. Aguirre[1], L Mendoza[1,3], M.L.J Bravo[1], L. Gusmão[4]

[1] Laboratorio GENES Ltda, Medellín, Colombia.

[2] Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

[3] Universidad Pontificia Bolivariana, Medellín, Colombia

[4] IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

Participation in exercises interlaboratory quality control is one of the main mechanisms currently used for quality assurance and continuous improvement of the trials. The objective of this study was to design, to manage and to evaluate the 2012 Colombian Quality Control Exercise (2012-CIQCE).

The 2012-CIQCE consisted of a mandatory and an optional section. The obligatory section, consisted in a theoretical and a practical exercise; for theoretical we sent the genotyping of 15 autosomal STRs markers of an alleged father and a son, the participants were asked to calculate the partial and total IP. For practical fragment three samples were provided, two from blood and one buccal swab, both on FTA cards, participants were requested to process the samples according to the methods and the markers used routinely in their own laboratories. The optional section consisted of three theoretical exercises of varying degrees of complexity.

This exercise involved 28 laboratories from 6 Latin American countries (Brazil, Ecuador, Peru, Panama, Dominican Republic and Colombia), all reported results for the theoretical obligatory and 27 for the practical. Fifty-five STR markers distributed in autosomal, Y and X chromosomes were under consensus. The Proficiency Test conducted through the Colombian National Reference Laboratory has become a useful tool for quality assurance of all Colombian laboratories and some of Latin America that do DNA testing to establish biological relationships, also is an excellent opportunity for constant experts training in the region.

Characterization of NIST Standard Reference Materials by Next-Generation Sequencing

K.M. Kiesler[1] and P.M. Vallone[1]

[1]National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, USA

The National Institute of Standards and Technology (NIST) offers certified Standard Reference Materials (SRMs) for laboratories performing DNA-based human identity testing. Developed by the Applied Genetics group at NIST, forensic DNA SRMs are well characterized for relevant markers such as autosomal and Y-chromosomal short tandem repeats (STRs) and mitochondrial DNA (mtDNA) sequence. NIST's forensic SRMs are required by the FBI Quality Assurance Standards for calibration of DNA typing instruments and procedures. Characterization of the SRMs thus far has been performed by capillary gel electrophoresis fragment-based genotyping analysis and/or Sanger sequencing. Ultra high-throughput sequencing, or next-generation sequencing (NGS), offers an opportunity to use an orthogonal method to further characterize DNA-based reference materials and thereby increase the confidence in the certified values of the materials. The additional sensitivity of NGS methods may increase the information content of the SRMs through the characterization of new features (e.g. SNPs, insertion-deletions, minor variants) within the markers' sequences which are beyond the ability of fragment-based analysis or Sanger sequencing to detect. This work will ultimately lead to certified reference materials for laboratories wishing to implement and validate NGS technology

in the field of forensics. Characterization of NIST forensic SRMs 2392 and 2392-I for mtDNA sequencing has been performed on several NGS platforms. Results of NGS mtDNA sequencing concordance with certified values determined by the Sanger method will be discussed. Preliminary data on sequencing of SRM 2391c for STR typing will also be presented.

A new frontier - international forensic science specific standards

L. M. Wilson-Wilde[1]

[1]Australia New Zealand Policing Advisory Agency National Institute of Forensic Science, Melbourne, Victoria, Australia

When applying international standards forensic science has worked towards to use of ISO17025, a standard designed for testing and calibration. Recent years has seen a move to the development of specific forensic science standards. In 2013 the first meeting of PC 272, an ISO committee for the development of international forensic standards, will be held. This heralds a new era with major implications for forensic science.

The first standard for development by the committee will be around minimizing the risk of contamination in products used to collect and analyse biological material for forensic DNA purposes. This standard is in response to numerous examples of DNA contamination by staff associated with the manufacturing of consumables used in forensic DNA analysis, such as eppendorf tubes and other plastic ware, which have occurred in many laboratories internationally. The most high profile incident of manufacturer based contamination is the 'Phantom of Gelhorn' case where over a 16 year period, millions of Euros and thousands of police hours were spent searching for a suspected female serial killer in Europe. The female DNA profile was matched to over 40 crime scenes in Germany, Austria and France and was later found to have originated from a female worker at a swab manufacturing company.

In Australia 27 incidents of manufacturer contamination have been detected over the last two years. Internationally this figure is much higher. The implications of this and other standards will be discussed.

Processing challenging casework samples: New protocols on the QIAsymphony SP/AS instruments

M. Scherer[1], B. Alsdorf[1], M. Breitbach[1], K. Matthaei[1], A. Schnur[1], N. Scholle[1], L. Bochmann[1], A. Prochnow[1], H. Engel[1]

[1]Qiagen GmbH, Hilden, Germany

The QIAsymphony SP/AS instruments allow fully automated extraction and PCR assay setup of forensic casework samples. We recently developed new extraction protocols that further optimized the performance and increase sensitivity. Binding of DNA to the magnetic particles was enhanced by introducing a heating step prior to mixing, resulting in Casework Advanced protocols. In addition, elution in small volumes has been improved by using a mineral oil overlay that eliminates any dead volumes that cannot be recovered and that typically lead to reduced overall yields with decreasing elution volumes. The oil overlay is only used during the elution process, but there is no transfer of oil into the final eluate. These High Efficiency (HE) protocols now are available for 30 – 80 µl volumes. With the release of a new software package, normalization of STR PCR setup was introduced to the AS reaction setup module. The template amounts added to each reaction can be automatically adapted to a user defined target based on imported concentration data of samples. In order to save PCR consumables and time, minimal DNA template amounts can be set as well to automatically exclude samples from downstream STR analysis if no reportable result is to be expected. While this approach is economical, it requires a quantification method that is very sensitive and reliable to minimize any risk of false negative results. The Investigator Quantiplex Kits were developed to correlate well to the Investigator STR Kits or STR assays from other suppliers for samples close to the detection limit.

Re-certification of the NIST standard reference material 2372: human DNA quantitation standard

P.M. Vallone[1], E.L.R. Butt[1], D.L. Duewer[1], M.C. Kline[1]

[1]National Institute of Standards and Technology, Biomolecular Measurement Division, Gaithersburg, Maryland, USA

SRM 2372 was designed for use in the value assignment of human genomic DNA forensic quantitation materials. SRM 2372 consists of three human genomic DNA extracts in TE^{-4} buffer, each originally certified to have spectrophotometric absorbance of 1.0 at 260 nm. However, by five years after production the absorbance in all materials had increased to the point that the certified values were no longer valid.

Investigation revealed that the absorbance increases resulted from the slow conversion of double-stranded DNA (dsDNA) to single-stranded (ssDNA). The conventional conversion factor for dsDNA is 50 ng/µL per absorbance unit while that for ssDNA is 37 ng/uL. There was no evidence of any decrease in fragment size or change in behavior of the materials in numerous qPCR assays.

Since the materials remain fit for their designed use, the remaining SRM 2372 units have been recertified for the spectroscopic properties of ssDNA. Users interested in the spectroscopic properties of these materials are instructed to force complete conversion to ssDNA with sodium hydroxide (NaOH). Users interested in using the materials to benchmark qPCR assays should use the materials as supplied but note that use of the ssDNA measurement procedure resulted in the "Information values" for conventional mass concentration supplied in the SRM 2372 Certificate changing from 50 ng/µL to 60 ng/µL. Informational values for the total number of genome-equivalents ("copy number") present in the solutions also now provided, based on results from digital PCR assays of chromosomes 1, 4, 5, 6, 9 and 14.

DNA human identification for the family rights: one year of agreement between UNESP/Laboratory of Paternity and Public Defender Service in São Paulo State.

J.A. Martins[1], D.F. Braganholi[1], J.H.P. Felix[1], C. F. de Almeida[1], I. Brunelli[1], V.L.S. Alvarenga[1], R.M.B. Cicarelli[1]

[1]UNESP - São Paulo State University, School of Pharmaceutical Sciences, Laboratory of Paternity, Araraquara, São Paulo, Brazil

In Brazil, there is a high number of children with incomplete birth registration data. Our laboratory initiated the activities in March 2001 and recently, October 2011, signed an agreement with the Public Defender Service of the State of São Paulo (DPESP), involving to make DNA paternity tests for individuals without financial conditions but entitled to legal assistance living in a ratio of 100 kilometers from Araraquara region (Headquarters).

The laboratory offers different techniques including 21 autosomal (AS) short tandem repeat (STR) markers, 12 Y-STRs and 10 X-STRs. The methodology and reagents used in the examination are the consensual standards recommended for human identification. Each month, a list of the services provided is sent to DPESP for the payment.

After one year, 196 examinations were performed typing 605 people, including 165 complete triple (mother, son and alleged father); nine

duo (child and alleged father); twelve reconstructions (six solved by AS and X-STR and six by AS and Y-STR); four with mother, son, registry father and alleged father; six involving mother, two sons and alleged father, and one with alleged mother. Non-exclusions were obtained in 130 of 196 cases. A total of seven paternal mutations of one step were found: three to D18S51 and one to FGA, D8S1179, D2S1338 and D12S391. In conclusion, the Laboratory has a university outreach community service program provided by highly qualified human resources with a team including one PhD, 2 academic support fellows, two specialist students, one specialist professional and one coordinating professor.

J.H.P. Felix and C. F. de Almeida are supported by BAAEII-PROEX-REITORIA-UNESP.

D.F. Braganholi is supported by CNPq.

I. Brunelli is supported by CAPES.

A study of DNA analysis and ethical issues, incidental findings, DNA report and informed consent

S. Ferreira[1]

[1]Instituto de Pesquisa de DNA Forense – Polícia Civil do Distrito Federal, Brasília, Distrito Federal, Brazil

At the same time DNA analysis plays a crucial role in helping Justice in criminal and civil investigations, it arises many important issues regarding ethics, individual rights, social benefits, moral and legal questions, privacy and security of information. DNA analysis is used in paternity testing and also in criminal investigations such as human identification in disasters, armed conflicts and caseworks related to homicides and missing persons. In all situations, DNA testing may reveal incidental findings (IFs), genetic information not related to the scope of the original investigation, such as misattributed parentage and misattributed paternity. Besides IFs, other important issue to be discussed is the kind of information that should be present on DNA report and, consequently, be publicly available. The aim of this study is to discuss important issues that may arise from DNA analysis, the importance of the informed consent process and the consent forms and to contribute with some guidance to this subject. In this study, we discuss real situations related to IFs (disclosure and nondisclosure), kinds of response to individual requests and kind of information that should be on DNA report. For ethical reasons, social benefits and privacy, among other issues, policies of nondisclosure should be adopted. Relevant information should be on DNA report according to the informed consent. Policies of not including DNA profiles of participants on DNA report should be supported. Such data should be informed only in some circumstances like court requests and when it's important for the goal of the investigation.

Results of the 2013 relationship testing workshop of the english speaking working group

S.L. Friis[1], C. Hallenberg[1], B.T. Simonsen[1], N. Morling[1]

[1]Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

The English Speaking Working Group of the International Society for Forensic Genetics annually offers an exercise involving genetic analysis in a relationship case with the objective to compare the results obtained in the participating laboratories.

In 2013, the exercise included maternity testing of blood samples from two children and an alleged mother. The two children were a biological daughter and a granddaughter of the alleged mother. However, this information was not available for the laboratories. The samples were distributed to 56 laboratories together with a questionnaire and a paper challenge. The paper challenge contained typing results of a child and an alleged father. Database information about the allele distributions in the tested systems was provided, and the laboratories were asked to submit results of the biostatistical calculations and the formulas used. The paper challenge included autosomal and X-chromosomal STR data.

The presentation of the 2013 Relationship Testing Workshop will include concordance/discordance in typing results, collation of systems and kits used by the laboratories and an evaluation of methods and strategies applied for DNA-typing. Furthermore, the results of the biostatistical calculations of the paper challenge will be presented and discussed.

Evaluation of OSIRIS for forensic DNA casework and incorporation into laboratory work flow.

J. Sutton[1], T. Kalafut[1], R. Goor [2], D. Hoffman[2], J. Riley[2], L. Armogida[3]

[1] United States Army Criminal Investigation Laboratory

[2] National Center for Biotechnology Information, National Library of Medicine, Nat

[3] NicheVision Forensics, LLC

OSIRIS (Open Source Independent Review and Interpretation System) is a public domain software package developed by NIH for the analysis of .fsa and .hid files generated by the Forensic DNA analysis process. OSIRIS searches for peaks in an iterative fashion by fitting expected parametric data signatures to the observed data. Unlike traditional sizing methods for STR fragment analysis, OSIRIS does not use Southern methods to compare a sample to the ILS for base pair estimates. Instead, OSIRIS compares the ILS of a sample to the ILS of a ladder, and then does a direct comparison of the center of sample peaks to those of the ladder. OSIRIS incorporates numerous artifact elimination strategies to minimize the number of human edits required for casework samples. These advanced artifact elimination algorithms allow for a lower peak calling threshold than otherwise possible. This in turn allows for more information to be preserved from a sample, particularly in instances of lower level amplification. The USACIL developed and uses ArmedXpert software for all aspects of DNA casework other than the analysis of .fsa files. Because OSIRIS is open source, we set about the task of integrating OSIRIS into the ArmedXpert software we currently use as much as possible. Some advantages are that we can now work in one continuous software environment, are not as limited by noise and/or artifacts, and can use the OSIRIS engine in the more user-friendly environment of ArmedXpert.

How the automation of DNA data interpretation using ArmedXpert software has benefited the United States Army Criminal Investigation Laboratory (USACIL).

T. Kalafut[1], J. Sutton[1], L. Armogida[2]

[1] United States Army Criminal Investigation Laboratory

[2] NicheVision Forensics, LLC

The validation process required for a forensic DNA laboratory and its Technical Leader can often be described as a necessary evil. The end result ensures confidence in protocols and a robustness of the final product for reports and testimony. However, validation requires that time and resources are set aside from casework to process samples, review data, and generate output tables for conclusions. In addition, multiple software programs and

instruments are often needed for data analysis, which further complicates integrating all the necessary data. ArmedXpert is a software program initially developed and validated by our laboratory for interpreting DNA profile results in forensic casework. It is now commercially available through NicheVision. We have recently collaborated with NicheVision to create a validation module as part of the ArmedXpert software package. The end result is that the DNA Technical Leader is now able to complete validation projects in a much more efficient and timely manner. Specifically, this module helps with determining the analytical threshold for a particular instrument, peak height ratios, stutter products (plus, minus, and combined plus/minus), the effects of mixtures on stutter, and a stochastic threshold for both single source and mixed samples. The software determines these thresholds directly from importing laboratory empirical data using .fsa files or allele table data from both autosomal and Y-STR profiles. Concordance using any number of data tables generated by prior protocols or several protocols may also be evaluated for implementation. Finally, all necessary documentation for validation summaries is generated by the software.

Polish Genetic Base of Totalitarianism Victims

A. Ossowski[1,2], T. Kupiec[3], K. Szwagrzyk[2], J. Piątek[1], M. Stefaniak[2], M. Kuś[1], M. Bykowska[2], G. Zielińska[1], Ł. Szleszkowski[4], A. Thannhäuser[4], M. E. Jasiński[5], A. Binczak[1], A. Ciechanowicz[1], M. Parafiniuk[1].

[1]Pomeranian Medical University in Szczecin, Poland.

[2]Institute of National Remembrace, Poland.

[3]Institute of Forensic Research in Kraków, Poland.

[4]Wroclaw Medical University, Wrocław, Poland.

[5]Institute of Archaeology and Studies of Religion Norwegian University of Science and Technology Trondheim, Norway.

In this publication we want to present the Polish Genetic Base of Totalitarianism Victims. On 28th September 2012, the Pomeranian Medical University in Szczecin and the Institute of National Remembrance - Commission for the Prosecution of Crimes Against the Polish Nation signed off an agreement on the Polish Genetic Base of Totalitarianism Victims (PBGOT, www.pbgot.pl). Our aim is to use the latest methods of forensic genetics to identify the victims of totalitarian systems. The project assumes to obtain proper reference material from family members of victims of communist and Nazi crimes and due to the time passage this has become the most important task for PBGOT. Work includes also securing and storing biological material collected during the exhumation of the victims, and carrying the genetic identification process. The next task is collecting all available historical data of killed and missing people. All these procedures were already applied in practice during the first stage of identifying victims of communists buried on Powązki Military cemetery in Warsaw. At this cemetery hundreds of people were buried that had been killed through military court orders during the years 1948-1956. This number includes most of the members of the Polish resistance movement. In 2012, the first exhumations were carried out and resulted in finding remains of 111 people. A team of specialists in the fields of history, archeology, forensic anthropology, forensic medicine and genetics of several scientific units from all over Europe take part in this research. The main goal is to identify the victims and so far, remains of 11 exhumed people were identified.

Accreditation of a forensic genetics laboratory in Italy

U. Ricci[1], C. De Sanzo[1], I. Carboni[1], S. Iozzi[1], A.L. Nutini[1], F. Torricelli[1]

[1]AOU Careggi, SOD Diagnostica Genetica, Florence, Italy

Italy in 2009 acceded to the Treaty of Prüm, particularly important for the management of a forensic DNA database. Although DNA analysis is used in Italy since the beginning of the technique, the introduction of this legislation has an impact extraordinarily important for the quality of the results. In the law explicitly requires that laboratories authorized to acquire genetic profiling to feed the database are accredited according to the ISO/IEC standard. There are indications that the final will indicate in more detail the necessity of achieving ISO17025/2005 accreditation. In addition, this is specifically indicated in the Council Framework Decision 2009/905/JHA of 30 November 2009 and in the Recommendation 7 of ENFSI (European Network of Forensic Science Institutes).

Since December 22th 2009, ACCREDIA is the single Italian National Accreditation body appointed by the State to perform accreditation activity. ACCREDIA evaluates the technical competence and professional integrity of the conformity evaluation operators (laboratories and bodies), evaluating compliance with mandatory regulations and voluntary norms in order to ensure the value and credibility of the attestations they release. However there is no rule to define the ISO test method for the study of DNA polymorphisms in forensic. Thus, each laboratory should build their own analytical system that is robust, reliable and reproducible using its own system of internal validation. Here we discuss some points of the accreditation process that become essential to ensure the effectiveness and efficiency of the production process of genetic data to use correctly the nascent Italian DNA database.

Quality control in cases of identification

U.D. Immel[1], S. Lutz-Bonengel[2], R. Lessig[1] on behalf of the participating laboratories*

[1]Institute of Legal Medicine, Martin-Luther-University Halle-Wittenberg, Halle, Germany

[2]Institute of Legal Medicine, Freiburg University Medical Center, Freiburg, Germany

According to the Interpol standards, one of the primary methods to identify unknown persons is the forensic molecular analysis of DNA samples taken from the victims. In routine casework these victims often are found in a state of high decomposition. The quality of DNA preparations from such samples varies strongly and can be very poor. Following the recommendations of the ISFG bone samples should be investigated for DNA analysis. An important question is the quality of the results, as the analysis can fail, allelic drop out or drop in can be obtained.

Blind trials have been part of the quality control in several parts of the routine casework for many years. For example, such trials have been established by the GEDNAP (German DNA profiling group) for forensic stain analysis and by several societies of paternity testing. Such blind trials are also part of laboratory accreditation procedures. In mass disaster cases or cases of identification such quality exercises have not been established so far. Therefore, a German work group of the ISFG decided to establish a blind test using samples comparable to routine identification casework. The results of the first trial were reported in Vienna in 2011.

The second trial took place in 2013 using femurs collected from full body donors which had been stored under defined conditions for 8 weeks. In the following blind trial, 33 European laboratories analyzed a bone sample. The methods were not restricted. All laboratories could use their own established protocols and available systems. The results of this blind trial will be reported.

** European Study Group:*

U.D. Immel, S. Lutz-Bonengel, M. Apfelbacher, C. Augustin, K. Bender, G. Braunschweiger, C. Courts, J. Edelmann, C. Geiser, U. Germann, F. Heidorn, S. Hering, C. Hohoff, K. Hoppe, S. Hummel, S. Köhnemann, A. Kurz, T. Lederer, B. Mattig, N. Morling, M. Nagy, F. Neuhuber, T. Parsons, A. Pieper, C. Puers, J. Sanft, C. Schmitt, K. Schober, M. Steinlechner, C. Tiemann, N. von Wurmb-Schwark, P. Wiegand, A. Wiemann, R. Zehner and R. Lessig

A Good Practice Guide for the use of Forensic Genetics in Investigations into Human Rights and International Humanitarian Law Violations

V. Penchaszadeh[1], A. Gershanik[2], C. Vullo[3], W. Goodwin[4]

[1] Consultant, Argentina

[2] Asamblea Permanente Por Los Derechos Humanos, Argentina

[3] EAAF, Argentina

[4] University of Central Lancashire, UK.

A consequence of armed conflict and other situations of violence is that individuals go missing. The problem of the 'Missing' can have a severe impact on the relatives and friends that are left without information on the fate of their loved ones and can be a barrier to the peace building process following the cessation of hostilities. The reality is that in many cases the Missing have been killed, and only through identification of the remains can closure be made possible.

Forensic genetics is playing an increasingly important role in identification of human remains post-conflict. However, in many situations problems can be faced when attempting to implement genetics into the identification program, both legally where issues around data protection and consent are common and technically, where the challenges faced by large-scale programs in the recovery of remains, extraction of DNA, collection of reference samples and matching of the DNA profiles have to be met.

In response the Argentine Ministry for Foreign Affairs and Worship (MREC), which has a long established record of dealing with the issue of the Missing within Argentina, proposed that the UN should support general recommendations on best practices in the use of forensic genetics to establish the identity of human remains: this was adopted in Resolutions 10/26 and 15/5. The Resolutions requested States, intergovernmental and non-governmental organisations to provide information on best practices when using forensic genetics. In response the MREC has coordinated experts, both legal and forensic, within Argentina (including the Argentine Forensic anthropology Team (EAAF) and the Grandmothers of Plaza Mayo), Latin America and then worldwide, to draft a set of guidelines. These will be submitted to the UN for consideration for use as reference for forensic identification programs that involve identification of human remains resulting from serious violations of human rights and international humanitarian law, especially in contexts that lack the necessary legal and technical framework.

Establishing a New DNA Databasing Laboratory? A Proposed Holistic Model for a Modular, Scalable and Efficient Implementation Programme.

Y. Prasad[1], A. Carbonaro[3], A. Agostino[4], S. Schroeder[2], G. Stead[1]

[1] HID Professional Services, Life Technologies, Paisley, United Kingdom

[2] HID Professional Services, Life Technologies, Frederick, USA

[3] Life Technologies, Foster City, USA

[4] Life Technologies, Italy

By end of 2012, it is estimated that there were only 44 countries with DNA Databasing legislation with a combined total of 40 million samples loaded on them. There are, however, a large number of countries that are already on the path ahead towards passing DNA Database legislation and some of them are relying on turnkey solutions to build their capacity in relatively short span of time. This paper discusses a scalable and modular framework which empowers laboratories to expand their repertoire of expertise and build national DNA databasing laboratories in an integrated and comprehensive manner. This end-to-end solution starts from configuring and customising DNA collection device to forming a strict chain of custody of reference exhibits to be transferred to the laboratories via integration with LIMS to the DNA analysis. This particular workflow is cost effective, helps alleviate problems associated with cross-contamination and can deliver throughputs that are scalable to over 1000 samples per day per instrument. Instrument verification data generated in our laboratory using Copan NUCLEIC-CARD system and direct PCR amplification with Life Technologies STR kits such as the GlobalFiler kits and using an integrated imaging, punching and liquid handling platform (Hamilton easyPunch STARlet) will be presented in this paper. The importance of keeping a holistic view towards developing a national forensic DNA databasing infrastructure by combining the recommendations from various thought leaders and national/international accreditation bodies with that of professional consulting contracts with industry experts in designing, developing and commissioning in a co-ordinated and efficient manner will also be addressed.