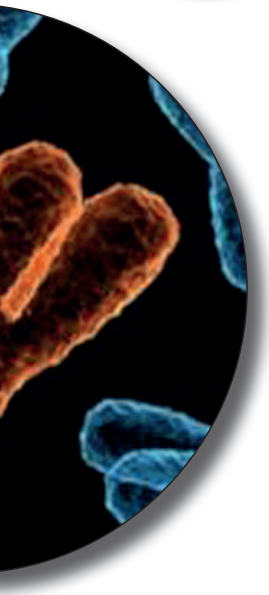

DNA in Forensics BRUSSELS 2014

9th International
Y-chromosome workshop

6th International
EMPOP meeting

Abstract book

14 - 16 May 2014 — Conference program

Abstract book

conference program

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DNA in Forensics 2014

9th Y-User Workshop and 6th EMPOP meeting

Dear friends, colleagues and guests,

We proudly present you the programme of DNA in Forensics 2014. All contributions, for which we are very grateful, will be presented in 56 oral communications and 61 posters.

The question “Is NGS NowGS in forensics?” will be answered by scientists who want to share their experiences on the latest sequencing technologies for various forensic applications: STR, SNP, mtDNA, mRNA and bacterial DNA.

The administrators of the two international Haplotype Population databases will announce how YHRD and EMPOP will deal with these new kinds of generated data.

Other items will be highlighted as well, such as Y and mtDNA population genetics, casework and its statistics, new Y-STRs and the impact of mutations rates, mtDNA variation, non-human DNA and ancient DNA.

This meeting will act as a forum to stimulate the interaction between participants, during the plenary session and in the coffee and lunch breaks as well.

Throughout this meeting, you will definitely pick up new ideas and widen your contacts in the forensic haplotyping community.

Let DNA in Forensics 2014 be your meeting,

Stijn Desmyter, Fabrice Noël and Sophie Verscheure, for the NICC organizing committee
Walther Parson and Lutz Roewer, for the scientific committee

The Belgian Institute for Forensic Science and Criminology (NICC/INCC) is hosting this event. We also acknowledge our sponsors and partners, illumina, Life Technologies, QIAGEN, Promega, VWR, Hamilton and BeBOL.

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Wednesday 14 May 2014

10h00 **Illumina Workshop**

13h30 **Opening Plenary session**

DNA in Forensics 2014 J. De Kinder

9th International Y-chromosome workshop L. Roewer

6th International EMPOP meeting W. Parson»

NGS

13h45 O01 Genome-wide profiling of STRs using high-throughput sequencing data
T. Willems, M. Gymrek, Y. Erlich (Cambridge, USA)

14h00 *O02* *Differentiation of Identical Twins using Next Generation Sequencing*
B. Rolf (Ebersberg, Germany)

14h15 *O03* *Forensic tissue identification via highly parallel sequencing of cross-species bacterial 16S rDNA with Ion Torrent PGM*
A. Ralf, O. Lao, D. Zubakov, M. Kayser (Rotterdam, Netherlands)

14h30 *O04* *Simultaneous human individual and tissue identification via highly parallel sequencing of STRs and mRNAs with Ion Torrent PGM*
D. Zubakov, I. Kokmeijer, A. Ralf, N. Rajagopalan, L. Calandro, S. Wootton, R. Langit, C. Chang, R. Lagace, M.Kayser (Rotterdam, Netherlands)

14h45 *O05* *Evaluating next generation sequencing technologies for expanded mitochondrial DNA identification capabilities at the FBI Lab*
J. Irwin, L. Moreno, T. Callaghan (Quantico, USA)

15h00 *O06* *Forensic evaluation of NGS technology on artificially degraded DNA samples*
I. Carboni, P. Fattorini, C. Previderè, C. Pescucci, M. Benelli, E. Contini, G. Marseglia, S. Bardi, F. Girolami, S. Iozzi, A. Nutini, U. Ricci, F. De Stefano, F. Torricelli (Florence, Italy)

15h15 *O07* *A new multiplex PCR system for forensic STR profiling using next generation sequencing*
K.J. Shin, E.H. Kim, I.S. Yang, S.E. Jung, H.Y. Lee, S.J. Park, S.H. Lee (Seoul, South Korea)

15h30 *O08* *Development of a Targeted Next Generation Sequencing Solution for Forensic Genomics*
K. Stephens, J. Varlaro, N. Oldroyd, C.Holt (San Diego, USA)

AGENDA

Wednesday 14 May 2014

15h45 O09 *Optimization of Two SNP Panels for Use with Forensic Samples on the Ion Personal Genome Machine®*
R. Lagacé, S. Wootton, C. Chang, R. Langit, J. Chang, N. Rajagopalan, L. Calandro (South San Francisco, USA)

16h00 O10 *QIAGEN's integrated GeneRead workflow for Next Generation Sequencing: from Sample to Insight*
K. Elliott, A. Prochnow, D. Mueller, R. Piest, T. Schnibbe (Hilden, Germany)

16h15 Coffee break

Non human DNA in Forensics

16h45 O11 *Reviewing dog mtDNA population studies for forensic purposes*
S. Verscheure, T. Backeljau, S. Desmyter (Brussels, Belgium)

17h00 O12 *Finding the right resolution*
M. Wesselink, I. Kuiper (The Hague, Netherlands)

17h15 O13 *MACE (MassiveAnalysis of cDNAEnds)-Highly sensitive digital gene expression profiling of Calliphora vicina (Calliphoridae) pupae*
B. K. Zajac, J. Amendt, R. Horres, R. Zehner (Frankfurt am Main, Germany)

17h30 O14 *Assessing the risk of incorrect identifications when DNA barcoding flies from forensic cases*
G. Sonet, K. Jordaens, M. Virgilio, Y. Braet, L. Bourguignon, E. Dupont, T. Backeljau, M. De Meyer, S. Desmyter (Brussels, Belgium)

17h45 O15 *An overview of the promises and pitfalls of the identification of flies (Diptera) of forensic interest using DNA sequence data*
K. Jordaens, G. Sonet, T. Backeljau, S. Desmyter, M. De Meyer (Tervuren, Belgium)

18h00 O16 *FORENSIC BOTANY II, DNA barcode for land plants: which markers after international agreement?*
G. Ferri, B. Corradini, F. Ferrari, S. Bertoncini, M. Alù (Modena, Italy)

NGS related large-scale projects

9h00	*O17*	*1,200 Y chromosome sequences from Phase 3 of the 1000 Genomes Project* C. Tyler-Smith, (Hinxton, United Kingdom)
9h30	*O18*	*Growing the Y-chromosome tree with 13,000 high-confidence SNPs from next-generation sequencing* M. Jobling, C. Batini, D. Zadik, J. Wetton, E. Arroyo, W. Bodmer, G. Cavalleri, P. de Knijff, G. Destro Bisol, B. Myhre Dupuy, L. B. Jorde, T. E. King, A. López de Munain, J. Milasin, A. Novelletto, H. Pamjav, M. Rocchi, A. Sajantila, W. Schempp, A. Tolun, C. Tyler-Smith, B. Winney, S. Watkins, P. Hallast (Leicester, United Kingdom)
9h50	*O19*	*Y-chromosome haplogrouping and paternal ancestry inference via simultaneous analysis of 550 Y-SNPs with IonTorrent PGM* M. Kayser, A. Ralf, K. Zhong, M. van Oven (Rotterdam, Netherlands)
10h05	*O20*	*In silico detection of phylogenetic informative Y-chromosomal SNPs from WGS data* A. Van Geystelen, T. Wenseleers, R. Decorte, M. J.L. Caspers, M. H.D. Larmuseau (Leuven, Belgium)
10h20	*O21*	*Seeing the wood for the trees: a minimal reference phylogeny for the human Y chromosome* M. van Oven, A. Van Geystelen, M. Kayser, R. Decorte, M. H.D.Larmuseau (Rotterdam, Netherlands)
10h35		Coffee break

Databases - Casework and its statistics

11h05	*O22*	*Next Generation YHRD: New Kits - New Interface - New Possibilities* S. Willuweit, L. Roewer (Berlin, Germany)
11h20	*O23*	*Application and statistic evaluation of Y-STR markers and mtDNA in forensic cases* J. Nagel, P. Dieltjes, P. de Vreede, M. Pouwels, R. Bink, A. Kal (The Hague, Netherlands)
11h40	*O24*	*Increased probative value in penetrative, spermatozoa negative sexual assualt cases using Y-STR analysis - a casework trial* E. Jones, J. Lewis, A. McDonald, P. O'Rourke (Abingdon, UK)

Thursday 15 May 2014

11h55	*O25*	*The combined evidential value of autosomal and Y-chromosomal DNA profiles obtained from the same sample* J. de Zoete, M. Sjerps, R. Meester, E. Cator (Amsterdam, Netherlands)
12h10	*O26*	*Match probabilities and the interdependence of Y-STR genotypes* A. Caliebe, A. Jochens, M. Krawczak (Kiel, Germany)
12h25	*O27*	*Analysis of Y-chromosomal STR population data using the discrete Laplace model* M. M. Andersen, P. S. Eriksen, N. Morling (Aalborg, Denmark)
12h40	*O28*	*The fundamental problem of Forensic Statistics* G. Cereda, R. Gill (Lausanne, Switzerland)
12h55		Lunch

New Y-STRs and impact of mutation rates

14h00	*O29*	*Forensic implications of the differential mutability of Y-chromosomal microsatellites* S. Siegert, J. Purps, L. Roewer, M. Nothnagel (Cologne, Germany)
14h15	*O30*	*A substantially lower frequency of uninformative matches between 23 versus 17 Y-STR haplotypes* R. Decorte, N. Vanderheyden, A. Van Geystelen, M. H.D., Larmuseau (Leuven, Belgium)
14h30	*O31*	*Development of an Italian RM Y- STR haplotype database: results of the 2013 GEFI collaborative exercise* C. Robino, A. Ralf, S. Pasino, M. De Marchi, M. Kayser (Turin, Italy)
14h45	*O32*	*Assessment of the number and quality of Y-STR systems and the effect of inbreeding on Y-STR profiling in genetically related populations – The Lebanese Model* M. Al-Azem, A. El Andari, S. Abbas, H. Othman, I. Mansour (Beirut, Lebanon)
15h00	*O33*	*Rapidly Mutating Y-STRs Multiplex Genotyping Panel to Investigate UAE Population* R. Alghafri, W. Goodwin, S. Hadi (Preston, United Kingdom)
15h15	*O34*	*A novel multiplex system amplifying 13 rapidly mutating Y-STRs* U. Rogalla, M. Wozniak, I. Dambueva, M. Derenko, B. Malyarchuk, J. Kubica, M. Koziński, T. Grzybowski (Bydgoszcz, Poland)
15h30		Coffee break

Thursday 15 May 2014

Y Population genetics

16h00 O35 *Searching for evidence of prehistoric gene flow from East Asia to South America*
Y. Xue, Y. Chen, M. Mezzavilla, M. Geppert, L. Roewer, C. Tyler-Smith (Cambridge, United Kingdom)

16h15 O36 *Association between Y haplogroups and AIMs revealed intra-population substructure in Bolivian populations*
C. Vullo, V. Gomes, C. Romanini, A. Oliveira, O. Rocabado, J. Gozi, A. Torres Balanza, A. Amorim, L. Gusmão (Córdoba, Argentina)

16h30 O37 *Fine scale Y chromosome analysis in Danes*
J. K. Olofsson, C. Børsting, N. Morling (Copenhagen, Denmark)

16h45 O38 *Low historical rates of cuckoldry in a Western European human population traced by Y-chromosome and genealogical data*
M. H.D. Larmuseau, J. Vanoverbeke, A. Van Geystelen, G. Defraene, N. Vanderheyden, K. Matthys, T. Wenseleers, R. Decorte (Leuven, Belgium)

17h00 O39 *Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma*
G. Barra, T. Santa Rita, C. Chianca, L. Velasco, C. Sousa (Brasília, Brazil)

Nuclear DNA

17h15 O40 *My-Forensic-Loci-queries (MyFLq) framework for analysis of forensic STR data generated by massive parallel sequencing*
C. Van Neste, M. Vandewoestyne, W. Van Criekinge, D. Deforce, F. Van Nieuwerburgh (Ghent, Belgium)

17h30 O41 *Identification of age-predictive epigenetic markers in forensically relevant body fluids*
H. Y. Lee, A. Choi, S. E. Jung, W. I. Yang, K. J. Shin (Seoul, South Korea)

17h45 O42 *Improving DNA Data Exchange: Validation Studies on a Single 6 Dye STR Kit with 24 loci*
P. Martin, L. Fernandez de Simón, G. Luque, M. J. Farfán, A. Alonso (Madrid, Spain)

20h00 **Social Event**

Friday 16 May 2014

AGENDA

EMPOP

9h00	*O43*	*EMPOP 3 - NGS mitochondrial databasing* *W. Parson, A. W. Röck (Innsbruck, Austria)*

mtDNA Population genetics

9h30	*O44*	*Increasing phylogenetic resolution of haplogroup U in Iberia through mtDNA complete sequencing* A. Goios, S. Marques, I. Nogueiro, A. Rocha, C. Alves, M. Prata, A. Amorim, L. Alvarez (Porto, Portugal)
9h45	*O45*	*Human settlement history of East Timor (Timor-Leste): NGS insights into the Pleistocenic diversity of mtDNA haplogroup P1* M. Bodner, S. Marcial Gomes, L. Souto, B. Zimmermann, G. Huber, C. Strobl, A.W. Röck, A. Achilli, A. Olivieri, A. Torroni, F. Côrte-Real, W. Parson (Innsbruck, Austria)
10h00	*O46*	*Further insights on the peopling of Greenland through the analysis of complete mtDNA sequences* V. Pereira, C. Børsting, N. Morling (Copenhagen, Denmark)

mtDNA variation

10h15	*O47*	*Frequency and Pattern of Mitochondrial Point Heteroplasmy in Human Tissues* J. Naue, S. Hörer, T. Sänger, C. Strobl, P. Hatzer-Grubwieser, W. Parson, S. Lutz-Bonengel (Freiburg, Germany)
10h30	*O48*	*Presence and variation of human mtDNA along and among hair shafts* S. Dognaux, G. Huber, S. Desmyter, C. Berger, F. Noël, W. Parson (Brussels, Belgium)
10h45	*O49*	*Heteroplasmic mutations throughout entire mitochondrial genomes of normal colon cells.* K. Skonieczna, B. Malyarchuk, A. Jawień, A. Marszałek, T. Grzybowski (Bydgoszcz, Poland)
11h00	*O50*	*Plasma sterilisation as a possible alternative to EO treatment in decontaminating laboratory consumables* M. Vennemann, K. Läer, M. Mozer, M. Schröder, F. Bittner, M. Klintschar (Münster, Germany)
11h15		Coffee break

Ancient DNA

11h45 O51 *DNA capture and Next Generation Sequencing recover whole mitochondrial genomes from highly degraded human remains*
J. Austin, J. Templeton, P. Brotherton, B. Llamas, J. Soubrier, W. Haak, A. Cooper (Adelaide, Australia)

12h15 O52 *Molecular structure analysis of an early medieval Alpine population using haplotypic markers*
M. Mayr-Eduardoff, C. Bauer, B. Zimmermann, G. Huber, C. Strobl, A. W. Röck, G. McGlynn, H. Stadler, W. Parson (Innsbruck, Austria)

12h30 O53 *The Use of mass screening toward the identification of 250 WWI soldiers from Fromelle*
P. Jones (Cambridge, United Kingdom)

12h45 O54 *The application of cloning techniques in the analysis of strongly degraded DNA samples*
C. Lischka, . S. Köhnemann, M. Vennemann, H. Pfeiffer (Münster, Germany)

13h00 O55 *Genetic research at a quintuple children's burial from medieval Cölln/Berlin*
J. Rothe, C. Melisch, M. Nagy (Berlin, Germany)

13h15 O56 *Preliminary results of the Collaborative exercise on DNA Typing of Bone samples*
D. Vanek, J. Dubska (Prague, Czech Republic)

13h30 **End of Plenary session**

13h45 Lunch

14h45 **Life Technologies "Advances in Lineage, Identity and Ancestry Informative Marker Analysis."**

Y-Chromosome

P01 *Analysis of the correspondence between the patrilineal family denominations and the Y-chromosome haplotypes in Sherpa population*
P. Tozzo, E. Ponzano, I. Amoruso, L. Caenazzo (Padova, Italy)

P02 *Developmental validation of the RM Y-STR multiplex system for casework and database samples*
R. Alghafri, W. Goodwin, S. Hadi (Preston, United Kingdom)

P03 *Analysis of mutation rates in father–son pairs using 13 RM Y-STR loci in the United Arab Emirates population*
R. Alghafri, M. Almarri, S. Alshehhi, W. Goodwin, S. Hadi (Preston, United Kingdom)

P04 *Internal Validation of a RM Y-STR Amplification panel for use in Forensic Casework*
R. Alghafri,S. Alshehhi, M. Almarri, W. Goodwin, S. Hadi (Preston, United Kingdom)

P05 *Y-chromosome short tandem repeat DNA typing of mixture samples from sexual assault cases using the PowerPlex® Y23 System*
J. J. R. Rodriguez, J. Salvador, G. Calacal, M. L. Honrado, R. Laude, M.C. De Ungria (Quezon City, Philippines)

P06 *Y-STR testing of 45-year-old bloodstained clothes soaked in miso*
K. Honda (Tsukuba city, Japan)

P07 *A collaborative project investigating more samples and more Y-SNPs to increase the knowledge on SA and CA Y chromosome diversity*
M. Geppert, L. Roewer (Berlin, Germany)

P08 *Evidence for extensive X-Y ectopic gene conversion in the evolutionary stratum 5 of human sex chromosomes*
B. Trombetta, D. Sellitto, R. Scozzari, F. Cruciani (Rome, Italy)

P09 *Phylogenetic refinement and SNP-based dating of human MSY haplogroup E*
B. Trombetta, E. D'Atanasio, A. Massaia, A. Coppa, G. Russo, A. Novelletto, R. Scozzari, F. Cruciani (Rome, Italy)

P10 *DYS392 bin 9: allele or artefact?*
B. Kokshoorn, K. van der Gaag, A.J. Kal, P. de Knijff (The Hague, The Netherlands)

P11 *Development and validation of the Yfiler® Plus PCR amplification kit, a new highly discriminating Y-STR Multiplex*
S. Gopinath, C. Chung, C. Zhong, V. Nguyen, M. Turnbough, J. Muehling, A. Carbonaro, J. Mulero (South San Francisco, USA)

POSTER

P12 *DNA analysis of deletion cases on Y chromosome in Jeju Island*

J. H. Lee, S. Cho, V. Shinde, D. Shin, S. Lee (Seoul, South Korea)

P13 *Y-STR genotyping of 32–56-year-old semen stains*
M. Hara, H. Nakanishi, A. Nagai, S. Takahashi, K. Yoneyama, K. Saito, A. Takada (Saitama, Japan)

P14 *Analysis of 16 Y-STR loci in a Japanese population*
A. Nagai, T. Ishihara, T. Takayama, M. Hara, Y. Bunai (Gifu, Japan)

P15 *Haplotype and mutation analysis for newly suggested Y-STRs in Korean father-son pairs*
K.J. Shin, Y.N. Oh, E.Y. Lee, E.H. Kim, W.I. Yang, H.Y. Lee (Seoul, South Korea)

P16 *PowerPlex® Y23 System: Internal validation of a new tool for Y-chomosomal genotyping of forensic casework samples*
A. Roseth, E. N. Hanssen (Oslo, Norway)

P17 *Croatian Y world - Always look on the bright side of Y*
I. Furac, M. Masic, M. Karija Vlahovic, M. Kubat (Zagreb, Croatia)

P18 *Genetic portrait of Lisboa immigrant population from Guiné-Bissau with Y-chromosome STR markers (preliminary results)*
A. Amorim, H. Costa, R. Reis, J. Lopes, F. Simão, T. Ribeiro, M. Porto, J. Costa Santos, C. Vieira da Silva (Lisboa, Portugal)

P19 *Genetic portrait of Lisboa immigrant population from Angola with Y chromosome STR markers (preliminary results)*
H. Afonso Costa, F. Simão, J. Lopea, C. Vieira da Silva, T. Ribeiro, M. Porto, J. Costa Santos, A. Amorim (Lisboa, Portugal)

P20 *Genetic Portrait of Cabo Verde native population living in Portugal with 23 Y-STRs- a preliminary study*
C. Vieira da Silva, A. Amorim, R. Reis, F. Simão, T. Ribeiro, M. Porto, J. Costa Santos, H. Afonso Costa (Lisboa, Portugal)

P21 *Haplotype diversity between PowerPlex Y and RM Y-STRs in a Portuguese population*
P. Brito, M. Oliveira, M. Carvalho, V. Bogas, H. Costa, A. Serra, A. Bento, F. Balsa, V. Lopes, L. Andrade, F. Corte-Real, M. Anjos (Coimbra, Portugal)

P22 *Male pedigrees: are RM-YSTRs useful to resolve a lineage?*
S. Turrina, S. Caratti, M. Ferrian, D. De Leo (Verona, Italy)

P23 *Comparing different population groups in Vietnam through Y-STR haplotype analysis*
F. Miranda-Barros, C. Romanini, L. Peréz, N. Nhu, T. Phan, E. Carvalho, C. Vullo, L. Gusmão (Rio de Janeiro, Brazil)

P24 *Application of Rapid Mutating Y-STR markers in forensic cases*
A. Kal, L. Clarisse, C. Van Kooten, L. Grol, T. Sijen (The Hague, Netherlands)

P25 *Familial Searching Combining Autosomal and Y chromosomal STRs and Surnames*
A. Kal,C. Van Kooten, R. Rintjema, J. Tjalsma, C. Reijenga (The Hague, Netherlands)

P26 *Genetic origin of the Kayah Karen in Northern Thailand: Evidence from bi-parental and paternal markers*
W. Kutanan, Khonkaen, Thailand

P27 *Central Asian admixture of paternal lineages among contemporary Hungarians*
H. Pamjav, T. Fehér, A. Bíró, G. Bárány (Budapest, Hungary)

P28 *Rearrangement of the AZF gene of the Y chromosome in the nuclear DNA extracted by male sporadic breast cancer tissue*
S. Gessa, M. Sestu, A. Carai, S. L. Meloni, F. Paribello, R. Demontis (Cagliari, Italy)

P29 *Can Rapid Mutant Y haplotyping be useful to solve cases of complex kinship testing?*
E. Carnevali, G. Margiotta, M. Lancia, S. Severini, L. Caenazzo (Padua, Italy)

P30 *Genetic polymorphism of 17 Y-chromosomal STR loci in the Kozha and Tore tribes of Kazakh population*
M. Zhabagin, Z. Sabitov, O. Balanovsky (Astana, Kazakhstan)

P31 *Amelogenin test abnormalities in men samples revealed during forensic DNA study and their interpretation*
S. Borovko, V. Korban (Minsk, Belarus)

Mitochondrial DNA

P32 *Mitochondrial DNA diversities and phylogenetic relationship of four major ethnic groups and Vedda population in Sri Lanka*
R. Ranasinghe, K. Tennekoon, E. Karunanayake, M. Allen, M. Lembring (Colombo, Sri Lanka)

P33 *mtDNA control region forensic database in the Romanian population and deep investigation of the most frequent haplotypes*
C. Turchi, F. Stanciu, A. Tagliabracci (Ancona, Italy)

P34 *The relation between the genetic mutations of mtDNA and sudden death cases with cardiac hypertrophy*
K. Maeda, C. Murakami, W. Irie, S. Nakamura, C. Sasaki, M.Oishi, N. Nakamaru, K. Kurihara (Sagamihara, Japan)

POSTER

P35 *Mitochondrial DNA landscape of Tunisia and its position within Mediterranean populations*
R. Kefi, S. Hsouna, L. Romdhane, K. Lasram, S. Abdelhak (Tunis, Tunisia)

P36 *Mitochondrial DNA data from the Netherlands to improve content and geographic coverage of the EMPOP database*
L. Chaitanya, M. van Oven, S. Brauer, B. Zimmermann, G. Huber, C. Xavier, W. Parson, P. de Knijff, M. Kayser (Rotterdam, Netherlands)

P37 *The association of POLG and p53 mutations with mitochondrial genome mutagenesis of colorectal cancer cells*
K. Linkowska, K. Skonieczna, B. Malyarchuk, A. Jawień, A. Marszałek, T. Grzybowski (Bydgoszcz, Poland)

P38 *Mitochondrial Point Heteroplasmy in Human Tissue Structures*
S. Hörer, J. Naue, T. Sänger1 S. Lutz-Bonengel (Freiburg, Germany)

P39 *In-solution targeted enrichment of the mitochondrial genome and forensically relevant nuclear SNPs*
R. Ellerington, W. Van de Voorde, R. Decorte, B. Bekaert (Guildford, United Kingdom)

P40 *Genetic portrait of Lisboa immigrant population from Angola with mitochondrial DNA (preliminary results)*
F. Simão, A. Amorim, C. Vieira da Silva, T. Ribeiro, M. Porto, J. Costa Santos, H. Afonso Costa (Lisboa, Portugal)

P41 *A new protocol to enable the analysis of inhibited mitochondrial DNA samples*
J. Harteveld, N. Weiler, L. Clarisse, T. Sijen (The Hague, Netherlands)

P42 *Polymorphism of the mitochondrial DNA control region in the population of Serbia*
S. Davidović, N. Kovačević Grujičić, M. Mojsin, V. Topalović, M. Stevanović (Belgrade, Serbia)

P43 *Full mtGenome reference population data: Development and evaluation of 588 forensic-quality haplotypes*
R. S. Just, S. Fast, M. Scheible, K. Sturk-Andreaggi, J. Bush, M. Peck, J. Ring, J. Higginbotham, E. Lyons, T. Diegoli, A.W. Röck, G. Huber, S. Nagl, C. Stroble, B. Zimmermann, W. Parson, J. Irwin (Dover, DE, United States)

P44 *Resolving the most common West Eurasian mtDNA control region haplotype in an Italian population sample by massively parallel mtGnome sequency*
M. Bodner, A. Iuvaro, C. Strobl, D. Pettener, S. Pelotti, D. Luiselli, W. Parson (Innsbruck, Austria)

P45 *Sequence polymorphism of the mitochondrial DNA hypervariable regions I and II in the population of Vojvodina Province, Serbia*
D. Zgonjanin, D. Drašković, S. Petković, R. Vuković, M. Maletin (Novi Sad, Serbia)

Non human DNA

P46 *Using DNA barcodes to identify forensic species of Diptera in Southeast Brazil*
P.V. Oliveira, F. de Paula Careta, J. Oliveira-Costa, G.G. Paneto (Alegre, ES, Brazil)

P47 *Coding region SNP analysis to improve dog hair mitochondrial DNA profiling for forensic purposes*
S. Verscheure, T. Backeljau, S. Desmyter (Brussels, Belgium)

P48 *A forensic database of cat mitochondrial DNA variants*
J. Wetton, B. Ottolini, G. Matharu Lall, M. Jobling (Leicester, United Kingdom)

Ancient DNA

P49 *Performance of the AutoMate Express for DNA extraction from old skeletal remains*
I. Zupanič Pajnič, B. Gornjak Pogorelc, K. Vodopivec Mohorčič, T. Zupanc, B. Štefanič, J. Balažic, M. Debska (Ljubljana, Slovenia)

P50 *Serial killers identified with DNA*
Z. Jakovski, R. Jankova Ajanovska, V. Poposka, B. Janeska, Al. Duma (Skopje, Macedonia)

P51 *Genetic analysis of bone samples - our experience*
C. B. Iancu, L. Barbarii, M. Rosu, D. Dermengiu (Bucharest, Romania)

P52 *The identification of Edward 'Ned' Kelly remains, some 130 years after his burial, and the role of mitochondrial DNA analysis*
D. Hartman, C. Vullo (Victoria, Australia)

Various

P53 *Estimation of homozygote/heterozygote drop-out probabilities and internal validation of a mixture profiling method*
I. Aladzsity, N. Mátrai, G. Tömöry, S. Füredi (Budapest, Hungary)

P54 *Population data and forensic parameters of 30 insertion/deletion polymorphisms of Cabo Verde immigrants in Lisbon*
R. Reis, P. Dario, T. Ribeiro, M. Porto, J. Costa Santos, A. Amorim (Lisboa, Portugal)

P55 *Genome-wide methylation profiling and a multiplex construction for the body identification using epigenetic markers*
H. Y. Lee, J. H. An, E. Y. Lee, W. I. Yang, K.J. Shim (Seoul, South Korea)

POSTER

P56 *Integrated Forensic DNA Data Analysis and Management - A scalable enterprise solution for forensic DNA laboratories*
A. Minn, J. Deng, J. Ge, M. Karpagavinayagam, N. Rajagopalan (South San Francisco, CA, USA)

P57 *Development Process Validation for Kinship Analysis Algorithm*
C. Dallett, S. Vijaychander, S. Rao, A. Swami, A. Minn (South San Francisco, CA, USA)

P58 *Stability of DNA stored in EO treated environment*
M. Vennemann, H. Holtkötter, M. Schürenkamp, U. Sibbing, H. Pfeiffer (Münster, Germany)

P59 *Expanding X-chromosomal forensic haplotype frequencies database: Italian population data of four linkage groups*
C. Bini, L. N. Riccardi, S. Ceccardi, F. Carano, D. Luiselli, S. Pelotti (Bologna, Italy)

P60 *DNAscan™ Rapid DNA Analysis™ System: Presentation of Data*
S. Ogden, J. French, E. Tan, R. Turingan, C. Hogan, S. Vasantgadkar, L. Palombo, J. W. Schumm, R. F. Selden (Cardiff, United Kingdom)

P61 *POWERQUANT™ SYSTEM: A NEW ROBUST HUMAN AND MALE SPECIFIC DNA QUANTIFICATION SYSTEM THAT MONITORS DNA INTEGRITY*
M.M. Ewing, J.M. Thompson, R.S. McLaren, A.J. Hopwood, S. Kutranov, D.R. Storts (Madison, USA)

PRESENTATION

001

Genome-wide profiling of STRs using high-throughput sequencing data

Short tandem repeats (STRs) are highly mutable genetic elements that form the cornerstone of modern forensic identification techniques. Despite their inherent utility, STRs have traditionally been characterized using low-throughput techniques such as capillary electrophoresis. To address this limitation, I will present lobSTR, a standalone tool to characterize STRs genome-wide from Illumina high-throughput sequencing data. We demonstrate lobSTR's accuracy by tracing Mendelian inheritance of STRs within a trio and by assessing the concordance of its calls with previous STR panels, including markers in the CODIS set and Y-STRs. In addition, we also demonstrate the utility of our tool by inferring surnames of anonymous genomes using genealogical databases and by characterizing STRs in whole-genome sequencing data.

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O02 Differentiation of Identical Twins using Next Generation Sequencing

Monozygotic (MZ) twins are considered being genetically identical, therefore they cannot be differentiated using standard forensic DNA testing. Here we describe how identification of extremely rare mutations by ultra-deep next generation sequencing can solve such cases. We sequenced DNA from sperm samples of two twins and from a blood sample of the child of one twin. Bioinformatics analysis revealed five Single Nucleotide Polymorphisms (SNPs) present in the twin father and the child, but not in the twin uncle. The SNPs were confirmed by classical Sanger sequencing. The method provides a solution to solve paternity and forensic cases involving monozygotic twins as alleged fathers or originators of DNA traces

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PRESENTATION

003 Forensic tissue identification via highly parallel sequencing of cross-species bacterial 16S rDNA with Ion Torrent PGM

Determining the cell / tissue type of biological materials used for DNA-based identification of sample donors can be of high relevance for crime scene reconstruction so that a battery of test systems were previously developed, most recently based on human m/miRNA. RNA-based identification of those forensically relevant tissues that naturally have high bacterial loads (e.g. vaginal secretion or saliva) may be improvable by use of bacterial DNA markers. Contrary to RNA markers, in principle, bacterial DNA markers do not suffer from technical problems caused by degradation and the absence of reliable quantification methods. However, previous attempts to support forensic tissue identification with bacterial DNA markers were complicated by the enormous inter- and intra-individual bacterial species diversity, which cannot be covered by a small number of diagnostic species applied before. To overcome this problem, we present here a non-species-biased bacterial DNA test system for identifying forensically relevant cell/tissue types. The wetlab part includes amplification of the 16S ribosomal RNA gene across bacterial species using conserved PCR primers, enzymatic shearing of amplification products to obtain fragments of the desired lengths (approx. 200 bp), and highly-parallel sequencing of the DNA fragments using the Ion Torrent Personal Genome Machine (PGM). Data analyses includes alignment of the obtained sequence reads with the E. coli reference sequence and generation of a distance matrix based on the relative nucleotide proportions of each position in a sample. This genetic distance matrix was then analysed via clustering algorithms to finally allow sample identification based on cell/tissue type. We demonstrate that this efficient and sensitive approach allows determining that a biological sample such as found at crime scenes originates from saliva, vaginal secretion, and fingermark traces (skin). This project was supported by Life Technologies.

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O04 Simultaneous human individual and tissue identification via highly parallel sequencing of STRs and mRNAs with Ion Torrent PGM

Individual identification of crime scene sample donors is usually performed via autosomal STR profiling, while determining the samples' cell or tissue type, informative for crime scene reconstruction, is recently achieved via mRNA profiling. However, both types of molecular analyses are currently performed separately, which not only is time- and resource intensive but potentially increases the risk of misidentification. We therefore developed a two-in-one test system that, based on highly parallel D/RNA sequencing using Ion Torrent PGM, delivers simultaneous individual and tissue identification from the same sample. After co-isolation of DNA and RNA from samples of all forensically relevant tissue types (saliva, peripheral and menstrual blood, semen, vaginal secret, and skin) from several individuals, the DNA and RNA fractions (1 and 10 ng, respectively) were processed separately. Total RNA was used to simultaneously amplify, using primers designed with the AmpliSeq RNA pipeline and barcodes across tissue samples, 12 mRNA markers (2 per each tissue type) previously suggested for forensic tissue identification as well as two house-keeping genes serving as controls and for normalization purposes. Total DNA was used to simultaneously amplify, applying an in-house protocol, 9 forensically used autosomal STRs as well as AMELX/AMELY for sex determination. The cDNA and gDNA amplification products were sequenced simultaneously with the Ion Torrent PGM by pooling barcoded individuals to further increase the analysis throughput while lowering the per sample costs. This proof-of-principle study demonstrates that highly parallel sequencing in general, and with Ion Torrent PGM in particular, is suitable to deliver a two-in-one single molecular test system that allows combining human individual identification with forensic tissue identification in a highly effective way and with a sensitivity required in routine forensic casework. Future developments will increase the number of STR loci included in the system to finally reach the full set currently applied in forensic practise.

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PRESENTATION

005 Evaluating next generation sequencing technologies for expanded mitochondrial DNA identification capabilities at the FBI Lab

Given the rapid developments in DNA sequencing technologies and the potential of Next Generation Sequencing (NGS) to not only increase the quantity and discriminatory power of genetic data but also improve the overall throughput of samples through the laboratory, the Federal Bureau of Investigation has recently initiated efforts to evaluate NGS. At this time these efforts are directed towards assessing the technology for its potential to expand current institutional DNA identification capabilities. Thus, an area of present interest is mitochondrial DNA (mtDNA) analysis, and the recovery of entire mitochondrial genome (mtGenome) data in particular. For our purposes, and from the standpoint of practical implementation in the long-run, we are evaluating methods that will efficiently yield robust mtGenome data from high quality specimens, while also considering approaches to the significant challenge of recovering entire mtGenome data from limited evidentiary specimens. Recent studies based on current technologies have shown that relatively large DNA fragments (>1kb) can often be recovered from the low DNA quality/quantity samples historically addressed with much smaller amplicons. We are therefore considering NGS assays that would incorporate this type of specific information. In the near-term, while developing methods and capabilities for mtGenome sequencing, we are also employing NGS to learn more about our samples. We hope to gain a better understanding of endogenous DNA quantity and quality, and by doing so gather additional information that can be used in the design of assays supporting human identification. We also envision that these data will offer insight into the possibility of treating select samples differently with currently established workflows. Here, we present an overview of our general efforts and the preliminary results of these studies.

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O06 Forensic evaluation of NGS technology on artificially degraded DNA samples

The main goal of this work is to test if Next Generation Sequencing (NGS) technology could be employed for retrieving genetic information useful for individual identification from degraded specimens not evaluable with conventional analytical protocols. The genetic substrate obtained from very degraded or quantitatively limited biological samples generally is in low amounts and very damaged in its primary structure; quite frequently, this genetic substrate comes from complex non recent casework, tested with pre-PCR methods (cold cases). These forensic specimens could represent important evidentiary sources for future criminal investigations. The aim of this project is to check the NGS technology on forensic samples for sensibility, reproducibility, reliability and error rate. The protocol is based on NGS analysis of a native DNA sample and of a set of depurinated DNA samples at different extents of degradation. NGS analysis will be performed using a custom enrichment kit (Agilent SureSelect XT, with a slightly modified protocol). The performances of the enrichment kit (that includes about 111 genes involved in hereditary cardiomyopathies and sudden death) have been evaluated analysing previously characterized clinical samples. The error rate and the coverage (a parameter that can be obtained from the number of reads of the same sequence, needed to verify the correspondence to the original sample) of the NGS will be verified by comparing the data obtained from the depurinated DNA samples to the reference DNA sequences. The results of this work could provide useful information on the reliability of NGS results obtained from forensic specimens.

In addition, since we are using a panel of genes relevant to hereditary cardiomyopathies and sudden death, the results of this project could help in setting up a clinical analysis of archive samples obtained from sudden death cases.

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PRESENTATION

007 A new multiplex PCR system for forensic STR profiling using next generation sequencing

Next generation sequencing (NGS) method has been tried to analysis short tandem repeat (STR) markers, which is mainly used for human identification purpose in the forensic fields. Some of the researchers demonstrated the possibility of successful application of NGS system to STR typing, while insisting that NGS technology may be an alternative or an additional method to overcome limitation of capillary electrophoresis (CE)-based STR profiling. However, there has been no available multiplex PCR system that is optimized for NGS analysis of forensic STR markers. Thus, we constructed a new single tube multiplex PCR system for the amplification of 18 markers (CODIS 13 STRs, and D2S1338, D19S433, Penta D, Penta E, Amelogenin), which contains amplicons in the size range of 70 to 210 base pairs. PCR products were generated from single-source and mixed samples using the multiplex PCR system developed in this study, and subsequent barcoded library were prepared for sequencing on the GS Junior system or the MiSeq system with obtained amplicons. Through performing NGS and analyzing NGS data, we confirmed that all the resultant STR genotypes were consistent with those of CE-based assay. Sequence variations could also be detected in targeted STR regions. In addition, the newly developed multiplex PCR system enables researchers to estimate mixture ratios successfully as well as to obtain STR allele calls effectively without missing of information at the STR loci which have large size amplicons in commercial kits. Therefore, the multiplex PCR system could be successfully applied to the STR analysis with single-source and even with mixed samples for forensic investigation using NGS system.

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O08 Development of a Targeted Next Generation Sequencing Solution for Forensic Genomics

Sequencing (NGS) by Synthesis (SBS) enables the entire human genome to be sequenced in one day. Whole genome sequencing (WGS) provides access to all genetic differences between individuals, and is valuable in studying disease and biological systems. While WGS delivers the broadest genomic coverage, it also requires the largest sequencing and interpretation effort. As a simpler alternative, forensic scientists can choose to perform targeted sequencing of PCR products. By sequencing a dense set of forensic loci, casework and database efforts are directed toward the genomic regions that best answer forensic questions, relieving privacy concerns and simplifying analysis. Because it does not depend on allele separation by size, the number of targets interrogated is not limited, allowing a more comprehensive result to be generated.

We describe the development of a targeted amplicon panel for forensic genomics that combines a core of global short tandem repeat markers used routinely today, along with additional forensic loci that can provide information when standard markers would fail to sufficiently resolve a case. Maximizing the number and types of markers that are analyzed for each sample provides more comprehensive and discriminating information for standard samples, as well as challenging samples that contain low quantities of DNA, degraded and/or inhibited DNA, and complex mixtures. The targeted amplicon panel will enable more complex kinship analysis to be performed, and can also reveal phenotypic and biogeographical ancestry information about a perpetrator to assist with criminal investigations. This capability is expected to dramatically improve the ability to investigate dead end cases, where a suspect reference sample or database hit are not available. We will describe the workflow, system, and data analysis tools, and present data from studies with challenging forensic samples, concordance with standard capillary electrophoresis methods, and possible kinship applications.

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PRESENTATION

009 Optimization of Two SNP Panels for Use with Forensic Samples on the Ion Personal Genome Machine®

Given rising interest in next-generation sequencing (NGS), forensic research has extended into areas previously limited by technology constraints. With the ability to genotype thousands of loci in parallel, NGS based SNP assays could become a valuable complement or alternative to STRs run on capillary electrophoresis. STRs can be sequenced alongside identity-informative and ancestral or phenotypic-informative SNPs, providing higher discriminatory power than STR kits and a statement of phenotype for investigative leads. We have developed two multiplex SNP kits, which have been refined under considerations for accuracy, coverage balance, and amplicon size for degraded DNA.

The identity-informative SNP panel comprises three subpanels. The two autosomal panels—Ken Kidd 45 unlinked SNPs and SNPforID 52-plex—consist of SNPs with high heterozygosity and low population heterogeneity, and Y-SNPs covering the major clades of the Y phylogenetic tree3. Ion Ampliseq™ chemistry was used to amplify and sequence the panel, and genotype concordance with TaqMan® was performed. Software to call genotypes and display supporting data was implemented. With desired >300x coverage for at least 90% of autosomal SNPs, 7 individuals can be barcoded and multiplexed on an Ion 314™ chip, 38 on an Ion 316™ chip, and 70 on the Ion 318™ chip.

The Ampliseq™ ancestry-informative marker kit includes two constituent panels. Kidd's 55 AIM SNPs and 128 markers from Seldin were used as the foundation for ancestral inferences. Software specific to this panel was developed to call genotypes and provide population statistics using the collective genotype on Kidd's ALFRED database.

Finally, each of these panels can be run in conjunction with compact STR panels to provide context with existing databases. The need to create standards for output and impose thresholds for quality and coverage is increasingly apparent in light of the outpouring of NGS data, and should remain a consideration throughout design and analysis.

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O10 QIAGEN's integrated GeneRead workflow for Next Generation Sequencing: from Sample to Insight.

Over the next decade Next Generation Sequencing (NGS) is set to become a fundamental component of forensic genetics, offering vast improvements to scientists and investigators over current techniques. Whilst currently still in the feasibility stage, in the future simultaneous analysis of multiple complex markers will unlock much greater content and enable the full potential of molecular information for human identification will be realized.

To achieve this level of sophistication in molecular analysis, NGS is necessarily complex, with numerous time-consuming steps and reactions. This presents challenges for implementation by the forensic community, which has become increasingly used to a relatively simple and highly automated process associated with current DNA profiling methods.

QIAGEN's GeneRead NGS workflow addresses these implementation challenges by encompassing the whole NGS process, from sample input through to analyzed and interpreted results. Here we present this workflow in detail, describing how pre-analytical steps (sample preparation, target enrichment and library preparation) have been automated using QIAGEN instrumentation and chemistry, whilst the sequencing step itself is conducted simply and cost-effectively using the GeneReader system. Finally QIAGEN's commitment to the bio-informatics challenges associated with NGS will be demonstrated by an overview of advanced bioinformatics tools (including CLC bio and Ingenuity), which together automate all steps from raw sequencing data analysis through to understanding the relevance of analyzed results.

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PRESENTATION

O11

Reviewing dog mtDNA population studies for forensic purposes

In the last 15 years, the identification of dog hair through mtDNA analysis has become part of forensic casework, as it may provide a link between victims and suspects. The evaluation of an mtDNA match between trace material and its potential donor depends on the haplotype's population frequency estimate. Importantly, this estimate has to be drawn from a population study representative of the population relevant to the forensic case. Numerous dog mtDNA studies have been published assembling control region sequences of over 8000 dogs worldwide. However, these studies vary widely in sampling strategies and data quality. This has led to the discussion which features influence the representativeness of a population sample. Moreover, several recommendations are made for performing and publishing a dog mtDNA population study for forensic purposes. In particular, reviewing the population studies indicated a need for the creation of a freely accessible database of high quality dog mtDNA population data.

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O12

Finding the right resolution

Species identification through DNA barcoding has gained great interest in the last years. Advocates of barcoding often emphasize the objectivity of DNA species identification and the fact a larger group of people is able to identify species using such techniques. Skeptics on the other hand point out that specialists are still needed to correctly describe and identify species and that algorithms for connecting a species name to a certain DNA barcode are still far from perfect, possibly resulting in incorrect species identifications.

We argue that in forensics, where it is extremely important to connect the correct name to a sample, a species name does not necessarily contain the relevant information. Determining which taxonomic level of identification is necessary to answer the forensic question, and which databases or references are needed to do so, will often prove more relevant.

We will demonstrate that for certain forensic questions the name of a genus or even a family contains more than enough information, making the distinction between species (or genera) irrelevant. Several examples of questions that can be readily answered with regular DNA barcoding markers will be presented, but also which drawbacks are inherent to using organelle chromosomes for answering such questions. Furthermore questions that are more problematic or even impossible to answer with barcoding markers will be described.

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PRESENTATION

O13 MACE (MassiveAnalysis of cDNAEnds)-Highly sensitive digital gene expression profiling of Calliphora vicina (Calliphoridae) pupae

Frequently, new methodological approaches in forensic sciences open up advanced perspectives that allow cases to be investigated more differentiated and faster. Such a novel approach is presented by MACE (MassiveAnalysis of cDNAEnds). Only cDNA-ends of transcripts are sequenced which leads to increased coverage. Each cDNA molecule is represented by one highly specific tag, originating from a 100-500bp region of the 3'end of a transcript. This achieves ultra-deep analyses to include the rare transcripts at about a 20 times lower sequencing depth as RNAseq. A typical transcriptome consists mainly of a few transcript species in high copy number, which can make up 40-80% of all transcripts, and many transcripts in low copy number, like receptors or transcription factors. These are often important for the understanding of crucial functions. MACE captures these low-level transcripts.

In order to break new ground in the field of forensic entomology, we analyzed the transcriptome of Calliphora vicina pupae at 15 different development stages. Determining a post-mortem interval using the weight or length of blow fly larvae is well established. But to date there are only a handful studies dealing with age estimation of blow fly pupae. From this study, we obtain new insights on the gene activity during metamorphosis. Genetic markers for molecular age estimation of pupae can extend the period for a successful post-mortem interval determination. We got 15 libraries with 3-8 million reads per library.53538 different transcripts were identified, 7548 were annotated to known insect genes.

The results help us to identify and understand the function of mRNAs included in metamorphosis which will assist in molecular age estimation of blow fly pupae. Relatively accurate data of gene expression facilitate selecting and increase the success of gene expression assays. Based on this study gene expression assays of new molecular marker for age estimation are being designed and tested.

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O14 Assessing the risk of incorrect identifications when DNA barcoding flies from forensic cases

The identification of fly larvae that feed on corpses can be decisive in forensics. For instance, different fly species may have different developmental times under identical conditions, which may lead to dissimilar estimations of the post-mortem interval. DNA barcoding is an identification method based on a short DNA fragment of the mitochondrial cytochrome oxidase subunit I gene (COI). Its application in forensic entomology is promising since specimens at all stages of their life cycle can be identified at the species level with a high resolution. However, this DNA-based identification relies on reference libraries of DNA barcodes (obtained for well-identified specimens), which might be incomplete (i.e. some species occurring in the investigated locality are missing) and might contain inconsistencies (e.g. different species with the same barcode). In order to assess the risk of incorrect DNA barcoding identifications, we assembled a local reference library with specimens from Belgian and French crime scenes. Then we used the new reference sequences as queries to assess the validity of the identifications provided by the available reference libraries. Although most queries could be correctly identified (>86%), some of them could not (no similar conspecific sequence in the library or more than one species assignment possible). In addition, we designed and tested a computer program allowing the user to perform DNA barcoding identification with a predefined relative error probability (e.g. 5%).

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PRESENTATION

O15 An overview of the promises and pitfalls of the identification of flies (Diptera) of forensic interest using DNA sequence data

The correct identification of fly larvae (Diptera) collected on corpses is one of the most important tasks in forensic entomology. The morphological identification of these larvae is often difficult, if not impossible. However, during the last decades, DNA sequencing has provided a wealth of information that can be used to identify larval stages of fly species of forensic interest. Here, we present an overview of 1) the currently available DNA reference sequences in public databases and 2) the statistical tools to assess identification success and reliability of fly identifications using DNA sequence data. We also illustrate several technological and biological aspects (e.g. hybridization, introgression, the occurrence of parasitic endosymbionts, recent speciation, population sub-structure, shortcomings of the databases, etc) that may affect the reliability of DNA sequence based species identification of these flies.

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O16 FORENSIC BOTANY II, DNA barcode for land plants: which markers after international agreement?

The ambitious idea of using short piece of DNA for large scale species identification (DNA barcoding) is already a powerful tool for scientist and the application of this standard technique seems to be promising in a range of fields including forensic genetics. While barcoding enjoined remarkable success for animal identification with cytochrome c oxidase I (COI) analysis, attempts to identify a single barcode for plants remained for longtime a vain hope. Until the beginning, the Consortium for the Barcode of Life (CBOL) showed the lack of agreement on a core plant barcode, reflecting the diversity of viewpoints. Different research groups advocated various markers with divergent set of criteria until the recent publication by the CBOL-Plant Working Group. After a four-year effort, the International Team in 2009 concluded to agree on standard markers promoting a multilocus solution (rbcL and matK), gaining the 70-75% of discrimination to species level. We firstly proposed in 2009 the broad application of DNA barcoding principles as a tool for identification of trace botanical evidence through the analysis of two chloroplast loci (trnH-psbA and trnL-trnF) in plant species belonging to local flora. Difficulties and drawbacks encountered include e the poor coverage of species in specific databases and the lack of authenticated reference sequences for the selected markers. Successful results were obtained providing a method and criteria to progressively identify unknown plant specimen to a given taxonomic rank, useful by any non-specialist botanist or in case of a shortage of taxonomic expertise available.

Now it seems mandatory to update and to compare our previous findings with the new selected plastid markers (matK+rbcL), taking into account the forensic requirements.

Features of all the four loci (the two previously analyzed trnH-psbA+ trnL-trnF and matK+rbcL) were compared singly and in multilocus solutions to assess the most suitable combination in forensic botany.

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PRESENTATION

O17

1,200 Y chromosome sequences from Phase 3 of the 1000 Genomes Project

The final phase of the 1000 Genomes Project has sequenced 1,244 males from Africa, Asia, Europe and the Americas at low (~4x) coverage and, via Complete Genomics, >200 at high coverage, as well as enriching for some Y-chromosomal regions. Sequencing is complete and the project is currently identifying Y-chromosomal SNPs, indels, STRs and structural variants. After validation, these will be combined into detailed haplotypes and used to construct a phylogeny. The high-coverage data include 30 father-son pairs sequenced from blood which will also allow de novo mutation rates to be estimated. We anticipate that the resulting dataset will offer powerful new insights into human evolutionary history and Y-chromosomal mutation and selection processes, which I will present.

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O18 Growing the Y-chromosome tree with 13,000 high-confidence SNPs from next-generation sequencing

The genetic variation of the paternally inherited male-specific portion of the Y chromosome (MSY) has been widely used in forensic analysis and in studies of human evolution; next-generation sequencing approaches can provide an unbiased picture of sequence diversity, which has been lacking until recently. We have sequenced ~4.4Mb of MSY to high coverage (~44X) in a total of 448 individuals. Among these, 374 were chosen on a population basis, mostly covering Europe and the Middle East, and the remaining 74 were selected with the aim of including all described major lineages of the MSY tree. A total of 13,261 high-confidence SNPs were identified and the resulting phylogeny resolves polytomies and provides date estimates of nodes, demonstrating a geographically widespread recent expansion affecting several major haplogroups. In addition, the population design allowed us to compare the histories of haplogroups across Europe, and to examine the geographical distribution of novel subclades. We also typed all samples with the Y-STR multiplex PowerPlex Y23, allowing a detailed comparison of haplotype diversity based on high-resolution STR typing with that from resequencing.

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PRESENTATION

O19 Y-chromosome haplogrouping and paternal ancestry inference via simultaneous analysis of 550 Y-SNPs with IonTorrent PGM

Obtaining high-resolution Y-chromosome haplogrouping and inferring detailed paternal bio-geographic ancestry, as relevant in forensics, genealogy and anthropology, requires large numbers of Y-SNPs that cannot be simultaneously analysed via conventional genotyping technologies. Therefore, by considering up-to-date phylogenic and geographic marker knowledge and applying state-of-the-art highly-parallel sequencing technology, we developed the MegaPlex-Y system for simultaneous genotype-by-sequencing analysis of over 550 carefully selected Y-SNPs using the Ion Torrent Personal Genome Machine (PGM). In addition to the wetlab protocol for generating the raw data, we also developed software tools for i) phylogeny-based quality control, ii) haplogroup determination, and iii) providing the most likely geographic region of haplogroup origin. As such, the MegaPlex-Y test system delivers an all-in-one solution for high-resolution Y-haplogrouping and detailed paternal ancestry inference. The system is highly sensitive delivering positive results from input DNA amounts of as little as 200 pg. We further demonstrate the feasibility, using barcode labeling, to combine multiple individuals in a single test run further increasing the analysis throughput while lowering the per sample costs. Given the high resolution of Y haplogrouping obtained from low input DNA amounts, and provided successful rigorous validation, we envision MegaPlex-Y to become the future tool of choice for high-resolution Y-SNP analysis in forensic, genealogy, and anthropology applications. This project was supported by Life Technologies.

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O20 In silico detection of phylogenetic informative Y-chromosomal SNPs from WGS data

«A state-of-the-art phylogeny of the human Y-chromosome is an essential tool for forensic genetics. The explosion of whole genome sequencing (WGS) data due to the rapid progress of next generation sequencing facilities is useful to optimize and to increase the resolution of the phylogenetic Y-chromosomal tree. The most interesting Y-chromosomal variants to increase the phylogeny are single nucleotide polymorphisms (Y-SNPs) especially since the software to call them in WGS data and to genotype them in forensic assays has been optimized over the past years. The PENNY software presented here detects potentially phylogenetic interesting Y-SNPs in silico based on SNP calling data files and classifies them into different types according their position in the currently used Y-chromosomal tree. The software utilized 790 available male WGS samples and in total 1,269 Y-SNPs potentially capable of increasing the resolution of the Y-chromosomal phylogenetic tree were detected with PENNY. Based on a test-panel we could prove that these newly added Y-SNPs indeed increased the resolution of the phylogenetic Y-chromosomal analysis substantially. Finally, we performed a second run with PENNY whereby all samples including those of the test-panel are used and this resulted in 509 additional phylogenetic promising Y-SNPs. By including these additional Y-SNPs a final update of the present phylogenetic Y-chromosomal tree which is useful for forensic applications was generated. In order to find more convincing forensic interesting Y-SNPs with this PENNY software, the number of samples and variety of the haplogroups to which these samples belong needs to increase.

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PRESENTATION

O21 Seeing the wood for the trees: a minimal reference phylogeny for the human Y chromosome

The human Y-chromosome phylogeny has important applications in human evolutionary genetics, genetic anthropology, genealogy, medical genetics, as well as in forensics where it can be useful to differentiate between potential male suspects and to infer the geographic region of patrilineal origin of a male DNA donor. The introduction of next-generation sequencing technologies has enabled, for the first time, large-scale sequencing of complete Y chromosomes, causing an avalanche of newly discovered Y-SNPs. The incorporation of these novel markers in the worldwide Y phylogeny is adding a huge layer of haplogroup resolution, thus allowing more precise phylogeographic inferences. However, the ever-expanding Y tree has grown to a level that it is hard to be practically comprehended in its entirety, while for many applications such ultra-high level of resolution goes beyond necessity. Furthermore, the 'complete' Y-chromosome phylogeny, including all Y-SNPs known so far, contains a considerable degree of redundancy because many Y-SNPs are phylo-equivalent (sit on the same branch). As a solution, we recently introduced a minimal version of the Y tree based on a strongly reduced set of Y-SNPs, aiming at optimal worldwide discrimination power. While we made sure to include all Y-SNPs needed to define the deeper (and thus older) branching structure of the Y tree, for the more recent radiations we selected only those Y-SNPs that have meaningful regional frequencies, following certain criteria. Furthermore, our tree aims to set a standard for Y-marker as well as Y-haplogroup nomenclature. The minimal Y tree (which should not be regarded as a replacement of complete Y phylogenies, which remain important for more detailed analyses) is meant as a convenient reference for a wide range of Y-chromosome researchers, and can be freely consulted at http://www.phylotree.org/Y.

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O22 Next Generation YHRD: New Kits - New Interface - New Possibilities

With the arrival of new highly informative YSTR kits (PowerPlex® Y23 System, Promega Corp. and Yfiler® Plus, Life Technologies) with 23 and 27 markers, respectively, a new way to present Y-STR data in the YHRD and to process queries is highly demanded. Currently all haplotype data must be manually typed to be queried. An alternative way of interfacing with the YHRD will be presented here, giving the user the possibility to upload haplotype data in various formats.

Furthermore we show a simplified but straightforward representation of matched / non-matched haplotypes together with advanced frequency calculations and match statistics. Options to further develop the YHRD from a pure database to an expert system for forensic Y chromosome application are discussed.

In summary we use those enhancements so show the simplicity of dealing with the new extended kit formats at the YHRD.

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PRESENTATION

O23

Application and statistic evaluation of Y-STR markers and mtDNA in forensic cases

At the Netherlands Forensic Institute (NFI) Y-STRs and mtDNA-analysis is routinely used in forensic (genetic) cases. Detection of the male component in DNA mixtures with a high female background is still the main field of application of forensic Y-STR haplotyping. Detection of mtDNA is applied in cases in which the autosomal DNA-concentration is low or non-existent, like e.g. in hairs. Another field of application is the use of Y-STRs and mtDNA to identify human remains. We will present data where Y-STRs and mtDNA were applied in both forensic cases and identification of human remains.

To evaluate evidentiary value of matches, YHRD(1) and EMPOP(2) is used as a basis to calculate LR values, using the estimated frequency of the haplotype. The calculation of LR values from matching lineage markers (Y-STRs and mtDNA) is done in several ways(3). Therefore standardized guidelines are desirable.

The NFI has developed a model to translate calculated LRs into verbal statements which are ultimately reported to court. This model is used institute wide, which helps to make reporting more consistent between different forensic fields of expertise and allows combining LRs from e.g. hair- and mtDNA-analysis.

(1)Willuweit S and Roewer L (2007), 'Y chromosome haplotype reference database (YHRD): Update', FSI:Genetics 1 (2), 83-7.

(2)Parson W and Dür A (2007), 'EMPOP-a forensic mtDNA database', FSI:Genetics 1(2), 88-92.

(3)Prieto L et al. (2013), 'GHEP-ISFG proficiency test 2011: Paper challenge on evaluation of mitochondrial DNA results', FSI:Genetics 7, 10–15.

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O24 Increased probative value in penetrative, spermatozoa negative sexual assault cases using Y-STR analysis – a Casework Trial

Over recent years the value of Y-STR analysis has become increasingly recognised by police forces in cases where there is a large amount of female DNA and very little male DNA. Although by no means limited to sexual assault investigations, this type of case is particularly suited to consideration for Y-STR analysis. In this study, Y-STR analysis was used to test vaginal swabs in UK sexual assault cases where penile or digital penetration was alleged, no spermatozoa were detected and swabs were taken within 48 hours of the alleged incident. The results of this casework trial show that Y-STR analysis can generate DNA profiles with significant probative value on these items, which will now be routinely considered for DNA analysis. Success rates and case examples will be given which illustrate the value of Y-STR analysis in these types of investigations.

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PRESENTATION

O25

The combined evidential value of autosomal and Y-chromosomal DNA profiles obtained from the same sample

When a Y-chromosomal and a (partial) autosomal DNA profile are obtained from one crime sample, and both profiles match the suspect's profiles, we would like to know the combined evidential value. To calculate the likelihood ratio of observing the autosomal and Y-chromosomal DNA profiles combined, we need to know the conditional random match probability of the observed autosomal DNA profile, given the Y-chromosomal match. We examine this conditional probability in two ways: (1) with a population genetic database containing 23 autosomal STR loci profiles and 17 Y-STR profiles of 2085 Dutch males, and (2) using a simulation model on family trees. We conclude that if the Y-chromosomal DNA profiles match, we can still regard the autosomal DNA profile as independent from the Y-chromosomal DNA profile if the matching person is not a descendant of the father of the donor of the (crime) sample. The evidential value can in that case be computed by multiplying the random match probabilities of the individual profiles. Recommendations on reporting combined autosomal and Y chromosomal data will be made.

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O26

Match probabilities and the interdependence of Y-STR genotypes

The calculation of match probabilities for rare Y-chromosomal STR profiles has been a subject of intense scientific research – and of hot, sometimes non-scientific debate. The issue is complex because haplotype population frequencies, the logical basis of match probability calculations under the 'random man' hypothesis, are usually difficult to estimate from representative databases if the number of STRs is too large and the corresponding haplotype diversity too high. Underlying this view is the assumption that Y-STRs are not statistically independent, owing to the lack of meiotic recombination, so that haplotype frequencies cannot be approximated by products of allele frequencies. However, the validity of this objection against the 'product rule' has never been assessed empirically. Since allelic association declines not only via recombination, but also through mutation, the nature of the statistical interdependence inherent to a specific Y-STR haplotype spectrum is only discernible from real life population data. We therefore analyzed the YHRD entries of the seven 'core' loci (19, 389i, 389ii, 390, 391, 392, 393) coming from four populations (Germany, Spain, China, Japan), with each sample comprising between 1700 and 4500 male individuals. Our analysis, based upon Shannon entropy, highlights that associations of all order exist between the seven Y-STRs, and that the uncertainty about a match at a given marker is reduced by no more than 50% through knowledge of the complementing (6-loci) haplotype. This implies that the problem of match probability calculation has no 'short cut' solution, for example, by the consideration of cleverly defined sub-haplotypes.

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PRESENTATION

O27 Analysis of Y-chromosomal STR population data using the discrete Laplace model

We present a new method based on the discrete Laplace probability distribution that approximates the distribution of short tandem repeat (STR) alleles when assuming a haploid Fisher-Wright model of evolution with a single-step mutation model. Both simulated data and real Y-chromosomal STR haplotype databases were analysed using the discrete Laplace method. The analyses could be performed on a laptop computer. The simulation study consisted of 9,000 data sets with 500, 1,000 or 5,000 Y-STR-profiles sampled from 60 different populations of size 20,000,000. The average deviation of the estimated probabilities of the Y-STR-profiles from the true population frequencies using the discrete Laplace method was smaller than those calculated with the naïve count estimate method (like 1/n or 1/(n+1) for data set size n) and Brenner's kappa method. When analysing real Y-STR databases, sound results were obtained, e.g. similar pairwise distances (between geographically separated sampling locations) to those obtained using the AMOVA method for a 7-loci European Y-STR database with approximately 12,700 males from 91 different locations and a 10-loci African Y-STR database with approximately 2,700 males from 26 different locations.

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O28

The fundamental problem of Forensic Statistics

When using Y-chromosome DNA profiles, it often happens that a DNA profile found on a crime scene and matching the suspect's profile does not appear in the relevant data-base. This creates a big challenge to the analyst who is required to supply a likelihood ratio (LR) or match-probability in order to quantify the evidential value of the match. Sensible estimation of the LR seems to rely on sensible estimation of the population frequency of this previously unseen haplotype.

There are three existing proposals of quite different nature: Roewer et al. (2000), based on Bayesian estimation of the haplotype frequency with a Beta prior; Brenner (2010), based on the number of singletons observed in the database; and Andersen et al. (2013) using a mixture of independent discrete Laplace distributions as a parametric approximation of the distribution of allelic frequencies.

We add two new methods. One is similar to Brenner's, and like Brenner's is strongly related to the Good-Turing estimator. A second method is based on Anevski, Gill and Zohren's (arXiv.org/math.ST:1312.1200) study of a non-parametric maximum-likelihood estimator. It is somehow intermediate between the parametric approach of Andersen and non-parametric methods based on Good-Turing estimators. We believe that it avoids the disadvantages of those while moreover providing a supplementary means of evaluating their accuracy.

For all methods it is imperative to assess two more levels of uncertainty, beyond the uncertainty about which hypothesis is true given the evidence, which would hold if we knew everything about the population probability distribution. LR is a ratio of probabilities which are usually based on a model which is at best only a good approximation to the truth. Moreover we only estimate parameters of that model by fitting it to the data in our database.

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PRESENTATION

O29 Forensic implications of the differential mutability of Y-chromosomal microsatellites

Background: Differential mutability of Y-chromosomal short tandem repeat (Y-STR) markers has been shown to be important for evolutionary inference of population parameters and forensic applications. Here, we investigate the impact of marker mutation rates on both standard forensic statistics and population substructure inference in a large global data set.

Methods: We analyzed previously published data of 19,749 Y chromosomes, sampled from 130 populations in 51 countries on five continents, that were typed for the 23 STR using the Powerplex® Y23 kit. We divided the marker panel into three sets of slowly, medium and highly mutating markers and compared resulting standard forensic statistics, such as haplotype frequency, haplotype diversity (HD) and discrimination capacity (DC), between these sets. Additionally, we performed an analysis of molecular variance (AMOVA) on the set-specific haplotypes in order to evaluate potential differences in the amount of detected population substructure.

Results: Increasing mutability of Y-STR markers led to an increase in the number of unique haplotypes per sample population and likewise to an increase in both HD and DC. While some population substructure was detectable with all three sets of markers, a strong differentiation was found for the set of slowly mutating markers. In particular, increasing mutation rates led to a decrease in the proportion of genetic diversity that was attributable to differences either among the 130 populations or among the five continental meta-populations.

Discussion: Our results indicate that the mutability of selected Y STRs has a substantial impact on both HD values and population substructure analysis. Thus, mutability of markers has to be taken into account when drawing population inferences.

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O30 A substantially lower frequency of uninformative matches between 23 versus 17 Y-STR haplotypes

The analysis of human short tandem repeats of the Y-chromosome (Y-STRs) provides a powerful tool in forensic cases for male sex identification, male lineage identification and identification of the geographical origin of male lineages. Since the commonly used 12/17 Y-STR multiplexes do not discriminate between some unrelated males, additional Y-STRs were implemented in the PowerPlex® Y23. However, the forensic value of a (near) 23 versus 17 Y-STR haplotype match between an unknown DNA donor and a certain biological sample in a database is not yet well studied. This will be of interest in cases where a database is used for familial searching or for the estimation of the geographical origin of the offender. In order to value (near) 23 Y-STR haplotype matches in a local sample from Western Europe, we used the region of Flanders (Belgium) due to the already present knowledge on its Y-chromosomal variants. Many Y-chromosomes of this region were previously genotyped with SNP's at a high resolution of the Y-chromosomal tree and the deep-rooted genealogy of each DNA donor was known. By comparing (near) matches of 23 versus 17 Y-STR haplotypes between patrilineal-unrelated males, a substantial lower number of uninformative (near) 23 Y-STR haplotype matches has been observed compared to 17 Y-STR haplotypes. Furthermore, the use of SNP data was informative to discriminate >60% of unrelated males with an (near) identical 17 Y-STR match while SNP data was only necessary to discriminate about 10% of unrelated males with a 23 Y-STR haplotype that differed at only two Y-STRs. This shows the higher value of the Y23 haplotype and, therefore, the use of the PowerPlex® Y23 System instead of the commonly used 12 and 17 Y-STRs by the forensic community is recommended as it will increase the efficiency of Y-STRs in forensic casework.

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PRESENTATION

O31

Development of an Italian RM Y- STR haplotype database: results of the 2013 GEFI collaborative exercise

Thirteen previously described rapidly mutating Y-chromosomal short tandem repeat (RM Y-STR) loci each displays a multiple-fold higher mutation rate relative to any other Y-STRs, including the markers conventionally used in forensic casework. Consequently, this RM Y-STR set has been demonstrated to improve the resolution of male lineage differentiation, and has been shown to allow male relative differentiation usually impossible with other Y-STRs [1]. However, large and geographically-detailed frequency databases are required to estimate the statistical weight of RM Y-STR haplotype matches if observed in forensic casework. With this in mind, the International RM Y-STR Study Group recently carried out a large multicenter study to collect worldwide RM Y-STR haplotype data [2], and subsequently the Italian speaking group of the International Society for Forensic Genetics (GEFI) launched a collaborative exercise aimed at generating an Italian national RM Y-STR haplotype database, presented here. Twenty-one laboratories -covering northern, central and southern areas of the Italian peninsula plus Sicily-, contributed to the project. After completion of a quality control step, participants analyzed local population samples (n≥50 per group), identified as "rural" or "urban" according to population density in the sampling area. Overall, >1000 haplotypes from twelve different Italian regional subpopulations were generated. In addition, each laboratory also provided haplotypes from a variable number of father/son pairs, for a total of more than 250 pairs, in order to refine previously established evidence for RM Y-STR based father-son pair differentiation. The results obtained will be discussed in terms of haplotype diversity, discrimination capacity observed at national/regional level, as well as between rural and urban areas, and male relative differentiation.

1. Ballantyne et al. (2012) Forensic Science International: Genetics 6, 208–218

2. Ballantyne et al. in preparation

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O32 Assessment of the number and quality of Y-STR systems and the effect of inbreeding on Y-STR profiling in genetically related populations – The Lebanese Model

Y-STR profiles are transmitted in haplotypes and when genetic relatedness is pronounced it may impose a limitation to the Y-STR applications. In Lebanon, consanguinity and endogamy average rates amount up to 36% and 88% respectively and could reach significantly higher rates in numerous rural areas where inbreeding is almost the rule. Thereby, it would be informative to assess the Y-STR profiling in the Lebanese population and determine the effect of these practices on the Y-STR profile match probability.

Y-STR allele and haplotype frequencies were estimated through two different haplotype sizes tested using the Y-filer Kit (Applied Biosystems); DYS19, DYS390, DYS391, DYS392, DYS393, DYS389I/II, DYS385a/b, DYS437, DYS438, DYS439, DYS456, DYS456, DYS448 and Y-GATA-H4, and the Y-23 Kit (Promega Corporation) which includes six extra systems: DYS576, DYS570, DYS549, DYS643, DYS533 and DYS481. 502 non-related males representative of the Lebanese population were sampled. In parallel, 148 males were sampled from four villages selected for their high inbreeding practices. In each village, at least 1% of the male inhabitants were sampled; (village 1 n = 12, village 2 n = 50, village 3 n = 41, village 4 n = 45).

With 16 loci, the 502 samples tested showed 463 different haplotypes, of which two were observed five times, three were observed three times, 25 were observed twice and 433 were observed once, reflecting discrimination capacity (DC) of 92.23% and haplotype diversity (HD) of 0.9995. With 23 loci, there were 490 different haplotypes, of which 478 were observed once and 12 were observed twice, reflecting discrimination capacity of 97.6% and haplotype diversity of 0.9999. The evaluation of the gene diversity showed that the DYS385a/b is the most polymorphic while the DYS392 is the least polymorphic. In villages, results showed very low discrimination capacities and haplotype diversities in all four villages as it follows: village 1 DC= 33.3%, HD= 0.470, village 2 DC= 68%, HD= 0.977, village 3 DC= 34.1%, HD= 0.839, village 4 DC= 53.3%, HD= 0.965. The most common haplotype was observed in village 3 and appeared as frequently as 14 times in 41 males, with extrapolation statistics yielding up to 45% of males possibly having this common haplotypes in this village.

In conclusion, the haplotype size and the quality of the included loci proved to be critical in the matching probability in the Lebanese population. There are very low discrimination capacities and haplotype diversities of Y-STR profiles among unrelated individuals belonging to the same village with high inbreeding practices even when using 23 Y-STR systems. The background is set to fully assess the Y-STR polymorphism in Lebanese sub-populations and to recommend more appropriate systems in this population.

Key words: Y chromosome, Y-STR, haplotype, frequency, consanguinity, Lebanese villages

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PRESENTATION

O33 Rapidly Mutating Y-STRs Multiplex Genotyping Panel to Investigate UAE Population

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. Recently rapidly mutating Y-STRs were described. These loci are expected to help investigating inbred populations and also differentiating closely related males. We have developed a multiplex panel 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 Department of Forensic Sciences and Criminology at Dubai Police. Thirteen, simultaneously amplified, markers included in multiplex are: DYF387S1, DYF339S1, DYF403S1ab, DYF404S1, DYS449, DYS518, DYS526I/II, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627. Four primer sets for DYF387S1, DYS570, DYS576 and DYS612 loci have been redesigned to accommodate the loci within the multiplex using 5-dye chemistry. An allelic ladder was developed using alleles found in UAE populations. 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.

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O34

A novel multiplex system amplifying 13 rapidly mutating Y-STRs

One of the major drawbacks of Y-STR analysis is the inability of the currently available systems to differentiate between closely related males. We have developed a multiplex amplification system that generates haplotypes for 13 Y-STR loci characterized by rapid or medium mutation rate ((DYS449, DYS458, DYS516, DYS518, DYS526b, DYS534, DYS547, DYS570, DYS576, DYS611, DYS612, DYS626 and DYS627). We have tested the novel amplification system in terms of both haplotype mutation rate and population polymorphism. Population diversity was assessed using samples from Central Europe as well as from the Middle East (Palestinian Arabs) and South Siberia (Buryats). We have demonstrated, that our system enables high resolution haplotype analysis even in populations consisting of closely related male lineages, such as Buryats.

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PRESENTATION

O35 Searching for evidence of prehistoric gene flow from East Asia to South America

The Americas were peopled from Siberia around 15,000 years ago, and appear to have had little subsequent contact with the rest of the world until colonial times. However, one of the largest surveys of Native South American populations thus far identified C3* Y chromosomes within a restricted area in Ecuador in the northern part of South America [Roewer et al. (2013) PLoS Genet. 4, e1003460]. This lineage is otherwise unknown in the Americas except in recent migrant populations, but is present at high frequency in parts of Central, East and North-East Asia. It is likely to have originated in Asia and been carried to South America later. Did this gene flow occur recently, or does it represent a previously unrecorded prehistoric event?

The Ecuadorian C3* chromosomes exhibit substantial diversity and prevalence in two nearby populations, with a common ancestor around 4,000 years ago. One of the populations carrying them experienced its first peaceful contact with outsiders in 1958 and previously discouraged contact using extreme ferocity, yet some members of this group born before 1958 carry C3* chromosomes. Archaeologists in 1962 suggested cultural contacts between the middle Jōmon culture of Kyushu (Japan) and a nearby region in Ecuador (6,400-5,300 years ago), based upon similarities in ceramics. Thus it seems possible that the C3* Y chromosomes were introduced into South America from East Asia in prehistoric times by sea, perhaps at the time of these cultural similarities.

We will present results from comparing genomewide autosomal SNP genotype data from these Ecuadorian samples with available East Asian and Native American populations to distinguish between prehistoric and recent gene flow. Thus this will provide novel genetic insights into a poorly-understood episode in the peopling of South America.

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O36 Association between Y haplogroups and AIMs revealed intra-population substructure in Bolivian populations

For the correct evaluation of the weight of genetic evidences in forensic context, it is important that databases reflect the structure of the population, with all possible groups being represented. Countries with a recent history of admixture between profoundly differentiated populations are usually highly heterogeneous and sub-structured. Bolivia is one of these countries, with a high diversity of ethnic groups and different levels of admixture (between native Amerindians, Europeans and Africans) across the country. For a better characterization of the male lineages in Bolivia, 17 Y-STR and 42 Y-SNP loci were genotyped in samples from the Andean population from La Paz and the sub-Andean population from Chuquisaca. Only European and Native American Y haplogroups were detected, and no sub-Saharan African chromosomes could be found. Significant differences were observed between the two samples, with higher frequency of European lineages in Chuquisaca (50.00%) than in La Paz (29.82%). A sample from haplogoup Q1a3a1a1-M19 was detected in La Paz, with a Y-STR haplotype background quite different from those previously found in Argentina. This result, at the same time supporting the hypothesis of a North-South spread of M19 in South America; also points to an old dispersion in two routes through the East and the West. Sub-structure inside La Paz and Chuquisaca was further investigated by comparing the ancestry of each individual assessed through his Y chromosome with autosomal AIMs. Increased European ancestry in individuals with European Y chromosomes and higher Native American ancestry in the carriers of Native American Y-haplogroups were observed, revealing that the association between autosomal and Y chromosomal persists after many generations of (potential) admixture. The results of this study demonstrate that sub-structure exist in Bolivia at both inter and intra-population levels, which must be taken into account in the evaluation of forensic genetic evidence.

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PRESENTATION

O37

Fine scale Y chromosome analysis in Danes

Previous studies have shown differences in Y-chromosome distributions between geographically close regions in Europe. Denmark's history and location in Europe makes it an interesting country for these types of studies. The aim of the present study was to investigate the Danish Y chromosome haplogroup (Y-HG) diversity in details.

A total of 179 male individuals from Denmark were analysed for 56 Y chromosome single nucleotide polymorphisms (Y-SNPs) and 17 Y chromosome short tandem repeats (Y-STRs). Four multiplexes (named Iplex, Qplex, R1aPlex, R1bPlex) defining sub-Y-HGs within the haplogroups I-M170, Q-M242, and R-P224, M207 were developed for the Sequenom MassARRAY® system. Haplotype diversity and the mean number of differences of each of the three major haplogroups (I-M170, R1a-M513, and R1b-M343) were estimated in Arlequin v. 3.5. Reduction median joining networks were drawn in Networks v. 4.6.1.1 and ρ -statistics were estimated.

Overall, 24 Y-HGs were found in the Danish population. The vast majority (90%) of the Danish individuals belonged to Y-HGs I-M170, R1a-M513 or R1b-M343, with only a few individuals belonging to other Y-HGs. Y-STR diversities were similar within the three major haplogroups I-M170, R1a-M513, and R1b-M343. The median-joining network method identified three less well-defined clusters corresponding to the three major haplogroups I-M170, R1a-M513, and R1b-M343.

This is the first study where the Y-HG diversity of the Danish male population was characterised in detail. This study is a first step towards an investigation of the Y-HG distribution on a micro-geographic scale in Denmark.

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O38 Low historical rates of cuckoldry in a Western European human population traced by Y-chromosome and genealogical data

Recent evidence suggests that seeking out extra-pair paternity (EPP) can be a viable alternative reproductive strategy for both males and females in many pair-bonded species, including humans. Accurate data on EPP rates in humans, however, are scant and mostly restricted to extant populations. Here we provide the first large-scale, unbiased genetic study of historical EPP rates in a Western European human population based on combining Y-chromosomal data to infer genetic patrilineages with genealogical and surname data, which reflect known historical presumed paternity. Using two independent methods we estimate that over the last few centuries, EPP rates in Flanders (Belgium) were only around 1-2% per generation. This figure is substantially lower than the 8-30% per generation reported in some behavioural studies on historical EPP rates but comparable to the rates reported by other genetic studies of contemporary Western European populations. These results suggest that human EPP rates have not changed substantially during the last 400 years in Flanders and imply that legal genealogies rarely differ from the biological ones. This result has significant implications for a set of promising forensic applications dealing with Y-chromosomes.

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PRESENTATION

O39 Fetal male lineage determination by analysis of Y-chromosome STR haplotype in maternal plasma

The aim of this study is to determine the fetus Y-STR haplotype in maternal plasma during pregnancy and estimate, non-invasively, if the alleged father and fetus belong to the same male lineage. The study enrolled couples with singleton pregnancies and known paternity. All participants signed informed consent and the local ethics committee approved the study. Fetal gender was determined by qPCR targeting DYS-14 in maternal plasma and it was confirmed after the delivery. The first consecutive 20 and 10 mothers bearing male and female fetuses, respectively, were selected for the Y-STR analysis. The median gestational age was 12 weeks (range 12-36). Peripheral blood was collected in EDTA tubes (mother) and in FTA paper (father). Maternal plasma DNA was extracted by NucliSens EasyMAG. All DNA samples were subjected to PCR amplification by ampFLSTR Yfiler, PowerPlex Y23 and an in-house multiplex, which together accounts for 27 different Y-STR. The PCR products were detected with 3500 Genetic Analyzer and they were analyzed using GeneMapper-IDX. Fetuses' haplotypes (Yfiler format) were compared to other 5328 Brazilian haplotypes available on YHRD. As a result, between 22 and 27 loci were successfully amplified from maternal plasma in all 20 cases of male fetuses. None of the women bearing female fetuses had a falsely amplified Y-STR. The haplotype detected in maternal plasma matched the alleged father haplotype in all cases. One case showed a mutation in the DYS438 locus, which was confirmed after the delivery. Seventeen fetuses' haplotypes were not found in YHRD and three of them occurred twice, which corresponded to paterninty probability of 99.981% and 99.944%, respectively. In conclusion, high discriminatory fetal Y-STR haplotype could be determined from maternal plasma during pregnancy starting at 12 weeks of gestation. Besides, all male fetuses could be attributed to the alleged father male lineage early in pregnancy.

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O40 My-Forensic-Loci-queries (MyFLq) framework for analysis of forensic STR data generated by massive parallel sequencing

Forensic scientists are currently investigating how to transition from capillary electrophoresis (CE) to massive parallel sequencing (MPS) for analysis of forensic DNA profiles. MPS offers several advantages over CE such as virtually unlimited multiplexy of loci, combining both short tandem repeat (STR) and single nucleotide polymorphism (SNP) loci, small amplicons without constraints of size separation, more discrimination power, deep mixture resolution and sample multiplexing.

We present our bioinformatic framework My-Forensic-Loci-queries (MyFLq) for analysis of MPS forensic data. For allele calling, the framework uses a MySQL reference allele database with automatically determined regions of interest (ROIs) by a generic maximal flanking algorithm which makes it possible to use any STR or SNP forensic locus. Python scripts were designed to automatically make allele calls starting from raw MPS data. We also present a method to asses the usefulness and overall performance of a forensic locus with respect to MPS, as well as methods to estimate whether an unknown allele, which sequence is not present in the MySQL database, is in fact a new allele or a sequencing error.

The MyFLq framework was applied to an Illumina MiSeq dataset of a forensic Illumina amplicon library, generated from multilocus STR polymerase chain reaction (PCR) on both single contributor samples and multiple person DNA mixtures. Although the multilocus PCR was not yet optimized for MPS in terms of amplicon length or locus selection, the results show excellent results for most loci. The results show a high signal-to-noise ratio, correct allele calls, and a low limit of detection for minor DNA contributors in mixed DNA samples. Technically, forensic MPS affords great promise for routine implementation in forensic genomics. The method is also applicable to adjacent disciplines such as molecular autopsy in legal medicine and in mitochondrial DNA research.

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PRESENTATION

O41 Identification of age-predictive epigenetic markers in forensically relevant body fluids

Age is an externally visible characteristic that is valuable for predicting individual's appearance in forensics. Therefore, age estimation based on molecular markers is expected to be useful to reduce a large group of potential suspects, when investigators cannot point out anyone as a suspect. Telomere length, accumulation of mutations and changes in gene expression are correlated with age, but the current most promising age-predictive biomarker is DNA methylation. Here, we analyzed DNA methylation patterns of 36 body fluid samples including blood, saliva and semen from individuals aged from 20 to 69 using the Illumina Infinium HumanMethylation450 BeadChip array. We identified CpG sites, whose methylation levels are strongly correlated with age and slope magnitudes are large with advancing years. Identified CpG sites were further tested for age prediction possibility using methylation SNaPshot methods in 40 more samples. Based on the results, we selected five or six CpG sites and built a regression model for age prediction in each body fluid. The model constructed for blood explained 89% of the variance in age with an average accuracy of 4.4 years. The model for semen explained 92% of the age variance with an average accuracy of 4.0 years. The model for saliva explained 50% of the age variance with an average accuracy of 10.0 years. Further development of multiplex system that enables the convenient quantitative analysis of methylation at selected CpG sites will facilitate the application of DNA methylation to forensic age estimation.

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O42 Improving DNA Data Exchange: Validation Studies on a Single 6 Dye STR Kit with 24 loci

The idea of the IDNADEX project (IDNADEX: Improving DNA Data EXchange, project HOME/2011/ISEC/PRUM/4000002125 awarded by the ISEC program) emerged in 2011 as an effective way to implement the new European Standard Set (ESS) of 12 STR markers adopted by The Council of the European Union in 2009, while maintaining an effective compatibility and information exchange with the historical DNA profiles contained in the Spanish national DNA database (around 200,000 DNA profiles at that time) mainly based on the 13 CODIS core STR loci plus D19S433 & D2S1338 markers. We proposed to test and validate a single STR amplification system for simultaneous analysis of 21 STR covering both CODIS and ESS core STR loci plus three additional markers (D19S433, D2S1338, and SE33) also contained in different STR kits and national DNA databases. We sought a global system with enhanced discrimination power for a better match efficiency that would reduce the chance of adventitious matches, and also showing optimal compatibility and concordance with the historical set of approximately 40 million of STR profiles registered in many national DNA databases around the world. This presentation describes the validation studies conducted with the first commercial available 6-dye STR kit for casework (now known as the Globalfiler PCR Amplification Kit) developed by Life Technologies in response to the CODIS Core Loci Working Group's recommendation to expand the CODIS Core Loci. This STR kit incorporates our proposal of 21 autosomal STRs (maintaining primer sequences of previous Identifiler®/NGM SElect™ kits except for the TPOX marker), the sex marker Amelogenin, and two new Y-chromosome markers: DYS391 & Y-indel. This study included the analysis of the following parameters and aspects: analytical threshold, sensitivity & stochastic threshold, heterozygous balance, stutter threshold, precision and accuracy, repeatability & reproducibility, genotype concordance, DNA mixtures, species specificity, and stability studies on case-type samples.

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PRESENTATION

O43

EMPOP 3 - NGS mitochondrial databasing

The emerging field of Massively Parallel Sequencing (MPS) also known as Next Generation Sequencing (NGS) has reached forensic genetics. It has been demonstrated that full mitochondrial DNA genome (mtGenome) sequences can be retrieved with forensic quality from degraded specimens that have so far only been successfully analyzed in the mtDNA control region with Sanger-type sequencing. Current research in forensic laboratories is evaluating the robustness and reliability of mtGenome sequencing using MPS technologies.

It is practice in routine forensic casework to search mtDNA sequences in databases in order to assess the rarity of a haplotype in a given population. With the advent of generating mtGenomes mtDNA databases need to be adapted to allow frequency estimates of (partial) mtGenome sequences.

We addressed this issue by developing a new version (Vs. 3) and website for EMPOP, the European DNA Profiling Group (EDNAP) Mitochondrial DNA Database (empop.org). EMPOP continues to perform sequence searches in unaligned format in order to guarantee that haplotypes are not missed due to different alignment of query and database sequences (string alignment method, SAM; Röck et al 2010). In addition, the haplogroup status of an mtDNA sequence is now provided via EMPOP by estimating haplogroups using maximum likelihood (EMMA; Röck et al 2013). This has an impact on the quality control of mtDNA sequences and provides the user with information on the phylogeographic background of a haplotype. Search results are displayed in tabular format and using geographical maps for finding matching haplotypes and for visualizing the global distribution of haplogroups to better assist the forensic practitioner in interpreting mtDNA evidence.

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O44 Increasing phylogenetic resolution of haplogroup U in Iberia through mtDNA complete sequencing

Human mtDNA has become an important genetic system in different fields, such as population, medical and forensic genetics, due to its unique characteristics, high copy number per cell and a maternal mode of inheritance, among others.

Accompanying the advances of the molecular biology techniques, the forensic filed has increased the detail of the analysis of mtDNA variation. Currently, sequencing of the hypervariable segments within the mtDNA control region is considered the standard analysis. However, complete sequences are more and more necessary to establish a correct phylogenetic assignment and for quality control of novel mtDNA data, although not yet routinely used in forensic casework.

In Western Europe and particularly in Iberia, the primary target for complete mtDNA analysis has been the most frequent (~40%) clade, H, particularly its sub-clades H1 and H3, because of the need to increase their phylogenetic resolution. However, other relevant haplogroups account for the mtDNA diversity of this geographic region. The second most frequent haplogroup is U (~10%), but at present there is a lack of complete haplogroup U sequences from Iberia in the available public databases.

In this work, we performed complete mtDNA Sanger sequencing of 35 U4 and U5 samples in 9 overlapping amplified fragments. Samples were selected from a previous analysis of 349 mtDNA control region samples from continental Portugal, where 10% of the diversity was found to belong to sub-haplogroups U4 (1.4%) and U5 (8.6%). We here present the complete genome sequences and analyse the increase in phylogenetic resolution of the mtDNA tree obtained for sub-haplogroups U4 and U5 in the Iberian peninsula.

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PRESENTATION

O45 Human settlement history of East Timor (Timor-Leste): NGS insights into the Pleistocenic diversity of mtDNA haplogroup P1

The details of human migration history in(to) today's Island Southeast Asia and Australia are still a matter of investigation. Several distinct, and partly competing, "waves" have been proposed based on non-genetic data and genetic markers, such as ages and dispersal patterns of particular mitochondrial DNA (mtDNA) haplogroups. The paucity of high quality complete mitogenomic data has impeded insights so far.

In this first representative study on the mtDNA variation of East Timor (Timor-Leste) population we focussed in particular on haplogroup P1, a poorly resolved autochthonous lineage of Island Southeast Asia and Melanesia in the Southwest Pacific region. We used the Ion Torrent Personal Genome Machine (PGM™) for generating complete mitogenome sequences in one of the first studies by stand-alone Next Generation Sequencing (NGS) approach to analyze a random population sample in a forensic genetic environment. We followed a strict validation strategy including double separate raw data analysis using different software. Our findings should contribute to the establishment of a standard practice in NGS mtDNA data validation that assures precise base calling - mirroring the accepted gold standard in Sanger-type sequencing.

Our results refined and greatly expanded available knowledge on the phylogeny of mtDNA haplogroup P1, revealed ample basal diversity including novel sub-clades beyond the solitary described, and allowed a more reliable coalescent age estimate for this lineage. The implications on the reconstruction of the initial migrations of anatomically modern humans into the region and insights into later processes from the composition of East Timor's extant mtDNA pool will be discussed.

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O46 Further insights on the peopling of Greenland through the analysis of complete mtDNA sequences

This study addresses the mtDNA genetic diversity in Greenlanders by means of Next Generation Sequencing of the whole mitochondrial genome. As a result of a complex settlement history that involved at least two Inuit groups, the Norse and the Danes from Europe, the present-day Greenlanders represent an interesting population with a genetic heritage that combines the components of both European and Inuit descent. Previous reports of lineage markers provided evidence of a sex-biased process of admixture between Inuit and European populations. We analysed complete mtDNA sequences of individuals from various regions of Greenland using the Illumina MiSeq Next Generation Sequencing platform and the Truseq® Nano DNA sample preparation guide for library set up. Sequences were classified into haplogroups according to the recommended nomenclature. Results showed a high predominance of haplogroups A2a and A2b, typically present in Inuit populations. This is in accordance with the major pre-European migration events. No European lineage was detected in the individuals analysed. Further statistical analysis and comparison with other populations were performed and will be presented.

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PRESENTATION

O47 Frequency and Pattern of Mitochondrial Point Heteroplasmy in Human Tissues

Mitochondrial (mt)DNA point heteroplasmy (PHP) is common in tissues of healthy individuals. The advent of new technologies revealed that its frequency has been underestimated in the past. Here, we strived for a comprehensive study investigating PHP in 9 different tissues from 100 individuals that underwent routine medical autopsy. The entire mtDNA control region of all samples was sequenced with the conventional dye terminator chain termination method (Sanger-type sequencing) and additional techniques were applied to confirm observed PHP including cloning, minisequencing and massively parallel sequencing. This study revealed that 88 % of the investigated individuals showed at least one PHP in any of the investigated tissues. In 75 % of these two or more PHPs were detected. PHPs were most abundant in muscle and liver, followed by brain, hair and heart. Lower values were observed in bone, blood, lung and buccal cells. Accumulation of PHP at specific positions was found in muscle, liver and brain. The presentation provides an overview of the observations in the light of sex, age, body mass index, tissue type and phylogenetic background of the individuals and highlights specific characteristics of the methods used.

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O48 Presence and variation of human mtDNA along and among hair shafts

The analysis of human mitochondrial DNA (mtDNA) in hair shafts found at crime scenes is an instructive part of forensic investigations.

The presence of sufficient mtDNA in an examined hair fragment is a prerequisite for a successful analysis. Quantification experiments on 249 hair shaft fragments originating from 20 hairs of 10 female individuals, between 24 and 36 years old, confirmed a decreasing number of mtDNA copies from root to tip. Furthermore, these experiments showed an intra and inter individual variation in mtDNA quantity.

The success rate for full control region Sanger sequencing of these quantified fragments was observed to be influenced by the hair color. These sequencing results revealed point heteroplasmic variation along the length of three individuals' hair shafts.

The intra individual variation of the mtDNA control region in hairs was further investigated through a second study. For 335 hair shafts, originating from three male and eight female individuals between 23 and 65 years old, a single fragment was analyzed by Sanger sequencing.

In total 68 point heteroplasmic variations were observed at 29 different positions, while two of the 11 individuals didn't show any variation. Five of the 10 homoplasmic variations were observed within a single individual.

No relation between variation frequency and hair colour/treatment, age or sex could be demonstrated.

Both experiments revealed differences between individuals in the frequency and the position of homoplasmic and heteroplasmic variations.

These studies give a better insight into the variation of mtDNA, which is helpful in case work with challenging profile interpretation.

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PRESENTATION

O49

Heteroplasmic mutations throughout entire mitochondrial genomes of normal colon cells

Next-generation sequencing technologies allow detection low-level mutations in DNA sequences. In this study we have employed 454-sequencing technology to detect low-level mtDNA variants in normal cells. We investigated variability of 50 complete mitochondrial genome sequences of normal colon tissues obtained from Polish colorectal cancer patients. Each sequence position was covered at least 1000 times. All mtDNA sequences were correctly assigned to the known mitochondrial haplogroups. 50% of investigated individuals were classified to H haplogroup, 30% to haplogroup U and 20% to clade JT. Heteroplasmic mutations were observed in 40% of normal colon specimens. Majority of the detected heteroplasmic mutations (about 80%) were observed at the level below 20% and thus would have passed undetected with traditional dideoxy sequencing. Out of 29 heteroplasmic positions identified, only three belonged to haplogroup diagnostic motifs. Most of the heteroplasmic mutations (62%) in normal cells hit positions observed in human mtDNA phylogeny. About 48% of heteroplasmic mutations were observed in non-coding regions, 38% localized in protein coding genes, 10% in rRNA genes and 3.5% in tRNA genes. Moreover, majority (71%) of the heteroplasmic substitutions located in protein coding genes lead to amino acid changes in the polypeptide sequence.

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O50 Plasma sterilisation as a possible alternative to EO treatment in decontaminating laboratory consumables

Ethylene oxide (EO) treatment is the current gold standard to remove possible DNA contaminations from consumables used for forensic DNA analysis, such as swabs, tubes and pipette tips. This technique, however, forms a serious health risk for personnel operating the decontamination device. Furthermore, residual EO may cause damage to trace DNA stored in materials which have been subjected to EO treatment prior to use.

In clinical settings, sterilisation with EO has been widely replaced by hydrogen peroxide plasma sterilisation. This technique uses vacuum to evenly distribute H2O2 in the sterilisation chamber before transferring it into the plasma state by high frequency. During the plasma phase, highly reactive oxygen species (ROS) cause severe damage to DNA with strand breaks and the formation of highly complex secondary structures. At the end of the decontamination cycle, H2O and O2 are formed as non-hazardous remains.

The aim of this study was to test the ability of the plasma procedure to remove DNA contamination from swabs, tubes and pipette tips. Extracted, high molecular weight DNA was transferred to different materials and the reduction rate of DNA after plasma sterilisation was calculated. Furthermore, cellular materials such as skin, saliva and blood were tested.

Plasma sterilisation was performed using the Sterrad 100S and 100NX instruments (ASP Johnson&Johnson, Hamburg, Germany) and the DNA contents were analysed using Quantifiler human DNA quantification kit (Life Technologies, Darmstadt, Germany).

Results show that DNA can be removed from various materials by plasma sterilisation. DNA amplification was not possible. Contaminations with cellular materials, however, seem to need a second decontamination cycle to fully remove traces of DNA.

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PRESENTATION

051 DNA capture and Next Generation Sequencing recover whole mitochondrial genomes from highly degraded human remains

Mitochondrial DNA (mtDNA) typing can be a useful aid for human identification in compromised samples when nuclear DNA is too damaged, degraded or below detection thresholds for routine short tandem repeat (STR)-based analysis. Standard mtDNA typing, focused on PCR amplicon sequencing of the control region (HVSI and HVSII), is limited by the resolving power of this short sequence which misses up to 70% of the variation present in the mtDNA genome. We sequenced whole mitochondrial genomes for human identification from samples where standard nuclear STR typing produced only partial profiles or demonstrably failed and/or standard mtDNA hypervariable region sequences lacked resolving power. Using in-solution hybridisation-based DNA capture (using DNA capture probes prepared from modern human mtDNA) we can recover mtDNA from post-mortem human remains in which the majority of DNA is both highly fragmented (<100 base pairs in length) and chemically damaged. The method 'immortalises' the finite quantities of DNA in valuable extracts as DNA libraries, followed by the targeted enrichment of endogenous mtDNA sequences and characterisation by Next Generation Sequencing (NGS). Multiple rounds of enrichment can substantially improve coverage and sequencing depth of mtDNA genomes from highly degraded samples. The application of this method has led to the reliable mitochondrial sequencing of human skeletal remains from unidentified World War Two (WWII) casualties ~70 years old and from archaeological remains (up to 2,500 years old). This approach has potential applications in forensic science, historical human identification cases, archived medical samples, kinship analysis and population studies. In particular the methodology can be applied to any case, involving human or non-human species, where whole mitochondrial genome sequences are required to provide the highest level of maternal lineage discrimination. Multiple rounds of in-solution hybridisation-based DNA capture can retrieve whole mitochondrial genome sequences from even the most challenging samples.

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O52 Molecular structure analysis of an early medieval Alpine population using haplotypic markers

Excavations conducted in Volders, Austria (Tyrol) in 1999-2000 revealed a medieval cemetery dated between the 5th/6th and 12th/13th centuries, which contained 141 human interments in 153 documented burials. Historically, the lower Inn Valley and its settlements, such as that found in Volders, played an important role as a thoroughfare between north and south. This was particularly evident during early Middle Age, a period in European history characterized by active migration and trade. Inhabitants of Volders are believed to have Raetish-Celtic roots. However, it is difficult to trace the origin of populations located at the cross-roads connecting different cultures to one single branch or lineage. In an attempt to shed some light on the genetic structure of medieval individuals from this area and compare it to a present-day population, we use molecular data from ancient individual remains, as well as recent DNA samples stemming from the same region and populations sharing an historical geographic association (Bavaria, Northern Italy). Using different population structure analysis methods (clustering, classification, Amova, PCA) we analyse mtDNA control region base composition profiles and Y-STR data to reveal intra- and interpopulation genetic differences.

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PRESENTATION

O53 The Use of mass screening toward the identification of 250 WWI soldiers from Fromelle

In 2010, 250 sets of remains from soldiers killed at the Battle of Fromelles were recovered and samples taken for DNA analysis in an attempt to identify as many as possible. In the process of the identification ca 1200 familial reference samples were taken and compared to the sets of remains. To date 119 individuals have been identified. I will describe the process of identification, the stability of the Y-STR and mitochondrial markers as well as some of the logical aspects of the projects that involved producing 1500 family trees with more than 3000 individuals mapped. The use of match probabilities in conjunction with other available identification information on the 5533 Australian and the 1547 British listed as missing will be discussed.

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O54 The application of cloning techniques in the analysis of strongly degraded DNA samples

We present a method to make strongly degraded DNA accessible to forensic analyses by the application of cloning techniques. Like almost all large molecules, DNA is affected by many different processes leading to its degradation. For example oxidizing chemicals, but also physical factors e.g. ionizing radiation, high temperatures or ultrasonics can cause severe damages to the DNA strand. Also biological processes as the activity of bacteria or fungi can degrade DNA. Especially when there is a large period of time between the crime itself and stain asservation, degradation phenomena are problematic.

All these degradation processes may lead to the problem, that DNA stains are no longer accessible to standard analysis methods like STR-typing. These techniques require DNA fragments of at least ~ 100 bp. Especially the loss of primer binding sites inhibits the analysis.

Cloning techniques are well-known in biotechnological research e.g. transfection of cells. Cloning requires the insertion of DNA fragment into a vector. We assume that this can help to make strongly fragmented DNA stains accessible to analyses. As a first approximation, artificially degraded DNA will be inserted into a vector, so common sequencing primers like T7 and SP6 can be used to get information on the inserted DNA fragment. We use a semi-quantitative approach to estimate the degree of DNA degradation. Then either the complete degraded sample or the fragments with the correct size, separated by gel extraction, are inserted into a cloning vector. A PCR of the bacteria colonies after transformation gives a first hint on the success of this method. Information on the recovered sequence might be helpful for the work of law enforcement authorities.

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PRESENTATION

055

Genetic research at a quintuple children's burial from medieval Cölln/Berlin

In the context of the medieval German eastward expansion into the former Germania Slavica many new cities were founded between the late 12th and 14th century. One of them is Germany's capital Berlin, which originated from the medieval twin cities Berlin and Cölln. Cölln was first mentioned in the records in the year 1237, but recent archeological findings in the area of former Cölln indicate a beginning of the settlement during the last quarter of the 12th century. Besides the uncertainty regarding the date of settlement foundation also the origin of the first settlers remains still unknown. Hereby, the analyses of written testimony are exhausted and only archeological research can help to generate new information. From 2007 to 2009 an excavation took place at St. Peters Square (Petriplatz) which is the former spot of the Cölln city center with St. Peter's church and its graveyard. The excavation revealed 3.717 skeletal individuals dating to the beginning of the 13th century till 1717 when the graveyard was closed for hygienically reasons. Except for the high number of excavated skeletons which represent a well preserved medieval population the excavation at Petriplatz showed a series of phenomena. It revealed for example an unusual high number of multiple burials with up to 12 individuals, carefully positioned in W-E direction. In order to test the DNA preservation of the skeletons from the Petriplatz, we first investigated a multiple burial with 5 children, who died at the age of 2 to 10 years. Our obtained genetic data of mt-DNA sequences and genomic STR markers clarified sex and kinship of the individuals. In addition with further archeological data genetic analysis contribute to shed more light on reasons for multiple burials and medieval funeral traditions. The received data give first insights into the genetic analysis of the Petriplatz burials.

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O56 Preliminary results of the Collaborative exercise on DNA Typing of Bone samples

«All participants of the Bone Workshop (Prague, Czech Republic, 2012) were able to participate in the Collaborative exercise on DNA Typing on Bone samples. Participating laboratories received 2 tubes with bone powder from 2 skeletons of different age (approx. 600 and 100 years) as well as the suggested protocol for DNA extraction. Those preliminary results are compiled from the results we received from 18 laboratories with 11 more still due to submit their results.

The presenting author will describe the sample preparation and comparison of different DNA extraction procedures and DNA typing results from participating laboratories including the following data:

- The amount of bone sample used for DNA extraction (in mg)
- Detailed DNA extraction protocol (including elution volume)
- DNA quantitation procedure and results (including sample curve when quantified by RT-PCR)
- PCR cycling protocol including the name of the amplification kit, PCR cycling protocol (number of cycles, PCR enhancers, etc.), name of the thermocycler AND /OR primers used for mtDNA sequencing analysis.
- DNA profiles (STR autosomal/gonosomal) and mtDNA haplotype (differences to rCRS)

Remark: The results are presented on behalf of participating laboratories.

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POSTER

P01 Analysis of the correspondence between the patrilineal family denominations and the Y-chromosome haplotypes in Sherpa population

In societies that use patrilineal family denominations, surnames and Y-chromosome follow a common pattern of inheritance and males sharing the same surname might also share the same haplotype in the nonrecombining segment of the Y chromosome. The Sherpa ethnic group, which inhabit in the South side of Himalayas, is divided into 18-23 clans denominated Ru, which is a term meaning “bones”. The male line transmits the Ru and so the father’s lineage determines one’s clan membership. This work is aimed at ascertaining if the Ru affiliation of Sherpa people corresponds to the family identification and denomination. Our aim was to establish the possible correspondence between the Ru and the Y-chromosome haplotype determined at 17 Y-STR in a sample of individuals belonging to this population, using the AmpFlSTR® YfilerTM PCR amplification kit. A mutational distance matrix was obtained and a Ru genealogical tree chart was drawn from it. A total population sample of 25 specimens was investigated in this study and 17 different haplotypes were detected. We conclude that the oral tradition of the Sherpas has generated a family identification system similar to that being used in societies with a formalized patrilineal transmission of surnames. The value of this study is also due to the characteristics of this population that is a good candidate for this kind of analysis. The data obtained in this study show that performing an anthropo-genetic research based on Y-STR haplotypes is a helpful and powerful tool at our disposal, in order to determine the true Sherpa Ru kinship and to confirm the genealogic tree that leads all of the eighteen major clans to the four original Sherpa’s proto-clans.

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P02 Developmental validation of the RM Y-STR multiplex system for casework and database samples

The Rapidly Mutating Y chromosome Short Tandem Repeat (RM Y-STR) System combines thirteen rapidly mutating Y-STR loci discovered recently (DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS518, DYS526I/II, DYS547, DYS570, DYS576, DYS612, DYS626, DYS627) . These loci have higher gene diversities than most of the loci in other commercial Y-STR analysis kits, allowing for further distinction between unrelated male individuals and also close relatives. The RM Y-STR System is designed to amplify DNA from purified extracts as well as direct amplification from FTA® cards used to collect database samples. Validation of the RM Y-STR System includes all of the studies required by SWGDAM revised validation guidelines. The results demonstrate that the RM Y-STR multiplex is a robust and reliable amplification kit capable of overcoming high concentrations of commonly encountered inhibitors such as hematin, humic acid, and tannic acid. Full profiles are consistently detected with 62.5 pg of male DNA, even in the presence of excessive amounts of female DNA, assuring the RM Y-STR multiplex as a sensitive method for Y-STR testing. Complete Y-STR profiles are detected from mixed samples with 500 pg of male DNA in a background of 1000 ng of female DNA.

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P03

Analysis of mutation rates in father–son pairs using 13 RM Y-STR loci in the United Arab Emirates population

The knowledge and precise estimation of the mutation rate in Y chromosome Short Tandem Repeats (Y-STR) is important for paternity testing and dating of Y chromosome lineage origins. In this study we examined more than 400 father-son pairs from the United Arab Emirates (UAE) using 13 Rapidly Mutating Y-STR (RM Y-STR) available in the recently developed RM Y-STR multiplex profiling system. Paternity confirmation, probability ≥99.9%, was performed using 15 autosomal loci. Our results provide empirical mutation rate estimates and demonstrate the necessity of both locus-specific and allele-specific estimates for forensic and population genetic purposes. Studying the mutational characteristics of the 13 RM Y-STRs has important forensic applications such as the definition of criterions for exclusions in paternity testing and the interpretation of genetic profiles in biological stain analysis.

POSTER

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P04 Internal Validation of a RM Y-STR Amplification panel for use in Forensic Casework

Y chromosome short tandem repeat (Y-STR) analysis has been conducted in the Department of Forensic Science and Criminology at Dubai Police since 2006 using the 17-loci AmpFlSTR®Yfiler® PCR amplification kit. In this study a Rapidly Mutating Y-STR (RM Y-STR) panel comprised of DYF387S1, DYF399S1, DYF403S1a/b, DYF404S1, DYS449, DYS528, DYS526I/II, DYS547, DYS570, DYS576, DYS612, DYS626 and DYS627 was validated following an internal validation outlined by the SWGDAM revised validation guidelines. We observe that 62.5 pg of male DNA generates a complete profile of the 13 RM Y-STRs (21 alleles) and as little as 31.25 pg of male DNA results in a partial profile at an average of 10 loci (18 alleles). In male-male mixtures, we detected a complete profile from the minor component in up to 1:3 ratios; while we observed most of the alleles of the minor component at a 1:9 ratio and more than half the alleles of the minor component at a 1:19 ratio. We obtained complete RM Y-STR profiles when 1 ng male DNA was mixed with female DNA at a ratio of up to 1:1000 (male:female).The RM Y-STR results obtained for adjudicated case samples gave significantly more probative information than normal autosomal and currently used AmpFlSTR®Yfiler® PCR amplification kit results. This study illustrates that RM Y-STR panel is extremely sensitive, does not exhibit cross-reactivity with female DNA, successfully types male DNA in the presence of overwhelming amounts of female DNA and is successful in typing actual forensic samples from adjudicated cases.

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P05

Y-chromosome short tandem repeat DNA typing of mixture samples from sexual assault cases using the PowerPlex® Y23 System

POSTER

DNA from evidence materials encountered in sexual assault cases are typically mixtures of victim and assailant DNA. Whereas autosomal STRs are powerful in human identification, complicated aSTR profiles resulting from these mixtures often pose challenges in interpretation. In the more common scenario where the victim is female and the offender is male, the analysis may benefit by additionally targeting male-specific Y-STRs in order to generate a single DNA profile that can be directly compared to a suspect's reference profile. This results to an increased power of including or excluding a person as a contributor to the DNA mixture.

PowerPlex® Y23 system (PPY23) co-amplifies 23 Y-STRs which include the 11 SWGDAM core Y-STR markers, 2 rapidly mutating Y-STR markers and 10 other Y-STR markers. We validated its suitability in an integrated DNA analysis workflow for sexual assault cases by testing its performance on simulated male-female and male-male DNA mixtures at various known proportions, and on DNA extracted from post-coital samples obtained from male-female and male-male couple volunteers. Results highlight the sensitivity of PPY23 to male DNA in male-female DNA mixtures and its value in complementing aSTR results with increased power of inclusion or exclusion. PPY23 can generate the full profile of the male component in at least 0.062:100 ratio of male-female DNA mixture, and the complete profile of the minor contributor in at least 1:19 ratio of male-male DNA mixture. Full profiles of a single male contributor were isolated in 25 out of 30 DNA extracts from vaginal swabs and stains obtained from the male-female couple volunteers. In contrast, only 2 out of 23 DNA extracts from male-male anal swabs and stains did not indicate presence of mixtures, which signify that mixture interpretation will likely be necessary in cases where the victim is male.

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P06

Y-STR testing of 45-year-old bloodstained clothes soaked in miso

On June 30, 1966 in Shimizu-city, Japan, a family of four was murdered, and their property burgled and set alight.

The suspect, Iwao Hakamada, a live-in employee and former professional boxer, was arrested because he could not provide a credible alibi.

The police stated that the suspect committed the crime wearing pajamas, but very little blood was found on Hakamada's pajamas.

Fourteen months later, five pieces of bloodstained clothing were recovered by police from a barrel at a miso factory. ABO blood typing matched Hakamada's blood type with that of blood taken from the shoulder of a miso-soaked shirt from the factory. Hakamada was found guilty and sentenced to death by the Shizuoka District Court. This sentence was upheld by the Supreme Court of Japan in 1980.

Hakamada consistently maintained his innocence and insisted he was falsely charged. DNA analysis was not completed because the state of the sample was so poor that STR typing using commercial kit produced little evidence in July, 2000. Hence, first requests for a retrial were rejected. The second application for a retrial commenced in April 2008 to allow DNA testing of clothing held in evidence. In August 2011, the defense team recommended Dr Honda conduct the DNA testing. Dr Honda successfully extracted DNA from the 45-year-old miso-soaked bloodstained clothes, and used the DNA for PCR and DNA typing using a novel technique (1, 2).

Dr Honda proved that the DNA extracted from the clothing did not match the Y STR profile (PPY 23) for Hakamada. This new evidence supports Hakamada's innocence, and it is hoped that Hakamada, at 78 years of age the world's longest-serving death row inmate, may be granted a retrial.

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(2) JFLM, 20 (2013) 326-327

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POSTER

P07 A collaborative project investigating more samples and more Y-SNPs to increase the knowledge on SA and CA Y chromosome diversity

In a recent collaborative study on South American (SA) Y chromosome diversity we analyzed more than 1000 indigenous individuals with up to 17 Y-chromosomal STRs and 16 Y-SNPs. The results showed that the genetic diversity of indigenous populations in SA is relatively low, with the majority of all individuals being designated to haplogroup Q and a low percentage being assigned to haplogroup C-M217. With the aim of differentiating the Q lineages and getting new insights into the earliest population history of SA two different individuals out of the >1000, both belonging to the subhaplogroup Q-M3, were analyzed with next generation sequencing. Several new candidate SNPs have been evaluated and 4 SNPs were confirmed as haplogroup Q specific and variable. One of the new SNPs is downstream to Q-M3 creating a new subgroup; the other three SNPs are upstream to Q-M3, describing branches at the same phylogenetic position or downstream to Q-M346. In the project presented here the collaboration is continued to focus on three main subjects. First, increase of the primary sample size, including also samples from Central America to investigate particularly the occurrence and distribution of the rarely found haplogroup C. Second, samples assigned to Q-M3 but none of its subgroups are analyzed for the newly described Y-SNPs and for recently published SNPs that also describe potential subgroups of Q-M3. Third, the phylogenetic position of the other three new SNPs is validated in relation to the constantly increasing number of subgroups of haplogroup Q. Therefore, a SNaPshot assay including two different multiplexes is set up. One multiplex includes 7 SNPs upstream to Q-M3 (M346, L54, CTS11969, Z780, Y14366724, Y14432230, Y15399584) and the second multiplex includes 6 SNPs downstream to M3 (M3, Y14998478, PV2, SA01, M19, M557). First results for an Ecuadorian sample set of Kichwa and Waorani are presented.

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P08 Evidence for extensive X-Y ectopic gene conversion in the evolutionary stratum 5 of human sex chromosomes

It has long been believed that the Male Specific region of the human Y chromosome (MSY) is genetically independent from the X chromosome. This idea has been recently dismissed due to the discovery that X-Y gametologous gene conversion may occur. However, the pervasiveness of this molecular process in the evolution of human MSY has yet to be exhaustively analyzed. In this study, we explored how pervasive X-Y gene conversion has been during the evolution of the entire stratum 5 of the human sex chromosomes. By comparing about 0.5 Mb of human-chimpanzee gametologous sequences, we identified 19 regions in which extensive gene conversion has historically occurred. From our analysis, it emerged that several of these hotspots are evolutionarily conserved between the two species. Furthermore, in order to explore the dynamics of X-to-Y non-allelic conversion in recent human evolution, we re-sequenced these nineteen hotspots in 68 widely divergent Y haplogroups, and found that at least six of them are still active in humans. Hence, the results of the interspecific analysis are consistent with the hypothesis of widespread reticulate evolution within gametologous sequences in the differentiation of hominini sex chromosomes. In turn, intraspecific analysis demonstrates that X-to-Y gene conversion may modulate human Y-chromosome-sequence evolution to a greater extent than previously thought.

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P09

Phylogenetic refinement and SNP-based dating of human MSY haplogroup E

Increasing the resolution of the human Y chromosome tree has led to a better temporal and spatial understanding of past population dynamics. Haplogroup E, defined by mutation M40, is the most represented human Y chromosome clade within Africa, and it is also found at relatively high frequencies across West Asia and Europe. To increase the level of phylogenetic resolution of this clade, we characterized by high-coverage (50x) next generation sequencing a set of 18 haplogroup E chromosomes. We identified a total of 559 variant positions, 76% of which novel. We constructed a maximum-parsimony Y tree and estimated the most recent common ancestor for all the nodes using a SNP-based approach. The phylogeny showed several novel features compared to the previous topology: the relative length of branches was drastically modified and the associated node ages changed. We used a subset of the variants here identified to genotype additional Y-chromosomes belonging to the E1b1b1-M35 sub-haplogroup. By this analysis, we resolved all previously known polytomies and assigned all the E-M35* chromosomes to different new monophyletic clades, thus increasing the discriminative power of the haplogroup for use in human evolution and forensics.

POSTER

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P10

DYS392 bin 9: allele or artefact?

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.

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P11

Development and validation of the Yfiler® Plus PCR amplification kit, a new highly discriminating Y-STR Multiplex

POSTER

Y-chromosomal markers have proven useful in solving investigations where low levels of male DNA are present in a high female DNA background. An intrinsic limitation of Y-STRs compared with autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome. Thus, in an effort to increase the power of discrimination we have developed a new 6-dye, 27-plex Y-STR system that includes the 17 markers from the AmpF® STR Yfiler® and Yfiler Direct kits 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® and Yfiler® Direct kits, the new multiplex shows improved performance in inhibited samples and admixed male and female samples at ratios >1:1000, better differentiation in male:male mixture samples in high female DNA background, and faster time to results. Additionally, 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.

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P12

DNA analysis of deletion cases on Y chromosome in Jeju Island

Mutation occurs in the Y chromosome, like other genes. However, unlike in autosomal genes the Y chromosome does not undergo recombination, so people in different areas may have different distribution patterns of Y chromosomal mutation. Detailed information derived from Y chromosomal mutation thus might provide further information on personal identification or phylogenetic history. In Y- STR tests on 668 habitants of the Jeju island, which is the largest island in Korean peninsula, null alleles at DYS448 were shown in 10 samples. We estimated the length of deletion by confirming specific sequence tagged site (STS) makers ranging from G66018 to sY1201 and we found that the pattern was similar with that of Kalmyks who is the historical ancestry of Mongolian. Historically, the Jeju island was governed by Mongolian for 100 years since 1273. We searched previous reports on Y chromosomal deletions and reviewed area-specific Y chromosomal mutations in the view point of regional difference.

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P13

Y-STR genotyping of 32–56-year-old semen stains

Aim: Y-chromosome specific-STR (Y-STR) genotyping of old semen stains has become important evidence in long-term unsolved sexual crimes. However, Y-STR-genotyping of very old samples has been little reported. In this study, we evaluated whether Y-STR genotyping of semen stains older than 30 years could provide useful forensic evidence.

Materials and Methods: Sixteen semen stain samples stored at room temperature (6, 32-39-year-old; 5, 40-49-year-old; 5, 50-56-year-old) were used. The acid phosphatase test was performed using SM test reagent and the samples were prepared for microscopy using the Baecchi staining method. DNA extraction and purification from the samples were performed with the DNA Extractor FM Kit (Wako, Osaka, Japan) using a 3 - 10 mg piece of sperm- stained gauze according to the manufacturer's protocol. Y-STR genotyping was performed using the AmpFlSTR Yfiler Kit (Applied Biosystems, Foster City, CA, USA) after DNA quantification using a Quantifiler Duo DNA Quantitation Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol.

Results: All samples showed positive reactions against the SM test reagent and sperm heads were observed microscopically in all samples. DNA contents detected by amplicon length of 130 bp were 253.8 ± 216.5 ng/mg in 32-39-year-old, 9.6 ± 11.3 ng/mg in 40-49-year-old and 7.9 ± 14.1 ng/mg in 50-56 year-old samples. The number of loci detected by Y-STR genotyping were 14.6 ± 0.4 (32-39-year-old), 9.2 ± 3.2 (40-49-year-old) and 9.4 ± 1.8 (50-56-year-old).

Conclusion: This study showed that Y-STR genotyping was effective with 32–56-year-old semen stains, suggesting that Y-STR genotyping can provide useful forensic evidence in long-term unsolved sexual crimes.

POSTER

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P14

Analysis of 16 Y-STR loci in a Japanese population

Allele frequencies and haplotypes for 16 Y-STR loci, DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438 and DYS448, were determined in a sample of 108 unrelated Japanese males living in Gifu Prefecture (central region of Japan) using AmpFlSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems). This population was demonstrated 106 haplotypes, of which 104 were unique, and two were found in two individuals. There was one haplotype with no allele detected at the DYS448 locus. The haplotype diversity value calculated from the 16 Y-STR loci was 0.9997. DYS385 showed the highest gene diversity value (0.9500), while DYS391 showed the lowest gene diversity value (0.2075). This new database of 16 Y-STR loci for Japanese population would be useful in forensic examinations and human genetic studies.

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P15

Haplotype and mutation analysis for newly suggested Y-STRs in Korean father-son pairs

In the present study, 363 Korean father-son haplotype transfers in 351 families were analyzed using an in-house multiplex PCR system for 14 Y-STRs (DYS385a/b, DYF387S1, DYS391, DYS449, DYS460, DYS481, DYS518, DYS533, DYS549, DYS570, DYS576, DYS627 and DYS643), which include 11 newly added loci to the PowerPlex Y23 system or the Yfiler Plus system. Each Y-STR showed gene diversities ranging from 0.2499 to 0.9612; high gene diversities were obtained for multicopy Y-STR loci, DYS385 and DYF387S1, with values of 0.9612 and 0.9457, respectively. Among a total of 351 haplotypes for 14 Y-STRs, 350 different haplotypes were observed with an overall haplotype diversity of 0.9999 and discrimination capacity of 99.72%. In 363 haplotype transfers from 351 pedigrees, 31 single step mutations were observed, and three father-son pairs showed mutations at two loci. Locus specific mutation rate estimates varied between 0.00 and 1.93® 10-2, and the average mutation rate estimate was 5.69® 10-3. Overall, mutations were frequently occurred at DYS449, DYS576 and DYS627 loci, which are known as rapidly mutating Y-STRs. The mutation rate estimates at most loci were not significantly different from those in other populations, but the mutation rate estimates at DYF387S1, DYS518 and DYS570 were somewhat lower in the Korean population than in other populations. In addition, DYF387S1 which has two-copies showed three alleles in eight father-son pairs and micro-variants were observed in 20 pairs at four loci (DYS449, DYS518, DYS570 and DYS627) without allele discrepancy between the father and son.

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POSTER

P16 PowerPlex® Y23 System: Internal validation of a new tool for Y-chomosomal genotyping of forensic casework samples

PowerPlex®Y23 System (Promega) is a recent commercial 23-loci, 5-colour Y-STR multiplex designed for genotyping forensic casework samples, database samples and paternity samples. Expanding the number of loci compared to other commercially available kits, it offers increased discrimination power in addition to higher sensitivity and robustness. For the internal validation we studied the repeatability, precision, sensitivity, contamination levels and male/male mixtures together with the interlocus balance and the level of stutters and pull-ups. The PowerPlex®Y23 System was also directly compared to AmpFlSTR® Yfiler® PCR Amplification Kit (Life Technologies) by performing parallel analyzes of forensic casework samples. Our validation results show a high degree of concordance with data published by Promega. However PowerPlex Y23 does not give considerably more genotype information for all samples in comparison to Yfiler.

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P17

Croatian Y world - Always look on the bright side of Y

This year we celebrate 20th anniversary of our forensic DNA laboratory. From the very beginning we have been using Y chromosome STRs for Croatian population study, relationship testing, deficient paternities, identification of missing persons or war victims and casework when many different males were involved.

Starting from singleplex reactions and PAGE back in 90s of last century to routine use of 23 Y-STRs and capillary electrophoresis we also witnessed the development of science and technology and their importance in forensics. In last decade more important improvement has been made in releasing commercial Y-STR kits than autosomal STRs even though Y chromosome typing results are not as informative as autosomal STR results.

Today, using a Y STR multiplex kits, male DNA typing is faster, better and cheaper. It is also more sensitive and easier to perform since there is no need for separation of male fraction in mixed stains like it was before. Besides progress every discovery brings up new and specific problems or challenges. In forensics the stress is on results' quality and interpretational issues.

Here we will show several selected examples from our lab and share experience from past and nowadays in using, upgrading and application of Y haplotype.

POSTER

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P18 Genetic portrait of Lisboa immigrant population from Guiné-Bissau with Y-chromosome STR markers (preliminary results)

At the end of 2010 the foreign population living in Portugal was about 445.000, i.e., about 4.2% of the total population. Particularly concerning to immigrants from Portuguese-speaking African countries, about 44 000 were from Cabo Verde, 23.500 from Angola and 20.000 from Guiné-Bissau. Immigrants from Guiné-Bissau, between 1991 and 2010, growth from about 3.986 to 20.000 (497%), and from those, about 16.000 are part of Lisboa immigrant population.

We are therefore dealing with a demographic reality completely different from that of the early 90's of the last century, not only at social and cultural level but probably also at genetic diversity level. Attempting to the new reality represented by the new Lisboa inhabitants our group intends to characterize them with genetic markers of population and/or forensic interest. In this study we present results of Y-STR typing.

Blood samples were collected from 50 individuals from Guiné-Bissau, presently inhabitants of Lisboa, and undergoing forensic investigations in INMLCF-DS. Y-STR typing was achieved with AmpFlSTR® YFiler® (AB). Haplotype diversity (HD) and pairwise genetic distances were calculated using the Arlequin software.

In the 50 male individuals from Lisboa immigrants of Guiné-Bissau sampled, we found 48 different haplotypes. From those 46 were unique and 2 with 2 records. The overall HD was 0.9979.

Our sample was compared with available population data on the YHRD, namely Northern, Southern and Central Portugal and Guiné-Bissau. Our sample of Lisboa immigrants from Guine-Bissau show significant differences with Guine-Bissau population of YHRD, which means that studied Lisboa immigrants from Guine-Bissau are not representative of their population of origin - Guine-Bissau -, or our sample is to short and further individuals need to be studied. As expected with the other three Portuguese populations, our sample showed significant differences.

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P19

Genetic portrait of Lisboa immigrant population from Angola with Y chromosome STR markers (preliminary results)

POSTER

In Portugal, and particularly in Lisboa, immigrant populations are growing as it happens all over Europe. By the end of 2010 the number of immigrants in Portugal are about 445 000. The migration contributes to increase social, cultural, religious, linguistic and anthropological heterogeneity.

One of the most representative countries of origin is the Angola with about 15.000 residents in Lisboa.

The aim of this study was to characterize paternal lineages of the Angolan immigrants who now inhabit in Lisboa as part of the population of southern Portugal, inferred by Y chromosome. To determine male lineages of the Angolan immigrants, 55 unrelated males, involved in routine forensic cases, were typed using the 17 Y-STR (included in the YFiler kit, AB). This study is enclosed in a research project conducted by Delegação do Sul do INMLCF to determine the actual genetic diversity of Lisboa population.

Genomic DNA was extracted from blood samples using the Chelex®100 protocol. The PCR amplification was carried out in a GeneAmp PCR 9700 Thernal Cycler (AB), according to the manufacturer's recommendations for the AmpFlSTR YFiler PCR Amplification Kit. Genotyping from DNA amplified products was carried out in an ABI PRISMTM 3130xl Genetic Analyser (AB) and using GeneMapper ID software v4.1. Haplotype frequencies and haplotype diversity (HD) were estimated using Arlequin software v3.5. The haplotype data were compared with previously published populations, namely Southern Portugal, Central Portugal, Northern Portugal and Guiné–Bissau. Analysis of molecular variance was performed using AMOVA tool provided by YHRD.

A total of 55 different haplotypes were found, all being unique. The overall HD was determined as 1.0000±0.0036. AMOVA results showed genetic distances between Angolan immigrants residents in Lisboa and the populations used for comparison. These results reinforce the importance of updating genetic data, including the new residents in the reference population, for forensic application.

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P20 Genetic Portrait of Cabo Verde native population living in Portugal with 23 Y-STRs - a preliminary study

Due to their paternal inheritance Y-STRs offers new perspectives for identification and kinship analysis and are also a precious tool in sexual assault cases with relatively high amount of female DNA and also mixtures from multiple male donors.

A newly developed Y-STR kit, Powerplex®Y23 system allows forensic geneticists to study 23 Y-chromosomal loci, all 17 markers included in the Y-Filer® kit, plus six additional markers: DYS481, DYS533, DYS549, DYS570, DYS576, and DYS643.

Cabo Verde is an African archipelago located in West Africa coast of the Atlantic Ocean discovered in 1460 by a Portuguese explorer, that found the islands uninhabited and without previous evidence of human presence, and according to historical literature colonization took place with caucasian Portuguese men and African slaves.

One sample of 50 individuals native from Cabo-Verde living in south Portugal with regional ancestry determined by recording genealogical information for one generation. DNA was extracted with chelex® method and amplification was performed using an Applied Biosystems GeneAmp® PCR system 9700 thermal cycler in a 12,5 µl PCR volume.

PCR products were separated by capillary electrophoresis ABI Prism® 3130 xl, and fragment analysis was performed with Genemapper® ID-X v 3.2, and alleles were determined by comparison to an allelic ladder supplied with the PowerPlex® Y23 kit.

Haplotype frequencies, gene diversity and Haplotype Diversity (HD) were estimated with Arlequin 3.5 software package and the results were according to what was expected. The discrimination capacity was also calculated by dividing the number of different haplotypes by the total number of individuals in the sample. Fraction of unique haplotypes was determined as the percent proportion of unique haplotypes using 23 and 17 Y-STR loci.

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P21

Haplotype diversity between PowerPlex Y and RM Y-STRs in a Portuguese population

Recent research with Rapidly Mutating Y-STRs (RM Y-STRs) have shown that these markers provide substantially higher haplotype diversity and haplotype discrimination capacity in worldwide populations when compared with the Y-STRs commonly used in genetic forensics. Contributing to the investigations that have been conducted, two different sets of markers were analyzed in a Portuguese population. The present study intends to compare the results obtained with PowerPlex® Y (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439) with those obtained with the set of RM Y-STRs previously analyzed (DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a + b, DYS626, DYS627, DYF403S1a + b, DYF404S1, DYS449, DYS547 and DYS612).

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POSTER

P22 Male pedigrees: are RM-YSTRs useful to resolve a lineage?

Analysis of Y-chromosomal short tandem repeats prove to be an extremely useful tool in forensic genetics to establish the paternal lineage, to discriminate the male component of DNA mixed stains (especially in rape cases) and in population genetics for evolutionary studies. Recently, the interest of the forensic community has focused on new Y-chromosomal short tandem repeats (Y-STRs), termed Rapidly-Mutating Y-STRs (RM-YSTRs), which may be able to differentiate between close male belonging to the same paternal lineage due to their high mutation rates.

In this work 67 pairs of male relatives from 30 father-son and 26 male brothers pedigrees related by 1-2 generations, originating from Northeast Italy, were analyzed with 13 RM-YSTRs in order to evaluate whether increased haplotype resolution could be obtained within a single lineage. The 26 separated male brother pedigrees were composed of 22 duos, 3 trios and 1 quartet that corresponded to 37 pairs of relatives.

Results showed that, in the 30 father-son pairs 9 mutations were detected, while in the 37 brother pairs 13 mutations were observed six of which found in three brother pairs. Overall, the most mutable markers were DYF399S1, DYS627, DYF403S1a and DYS570.

These findings suggest that further pedigrees studies on these markers are needed in order to establish their real suitability in forensic genetics.

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POSTER

P23 Comparing different population groups in Vietnam through Y-STR haplotype analysis

Vietnam is a multi-ethnic country subdivided into 58 provinces (divided into districts) and 5 municipalities.

In the present study we have collected 248 samples from unrelated males from four municipality and two province groups; four located in the Northern (Cao Bang, Ha Noi, Hai Phong and Lao Cai), one in the Central (Da Nang) and the other one in the Southern region (Ho Chi Minh). These samples were typed for the 17 Y-STRs included in the AmpFLSTR®YFiler® PCR Amplification kit with the aim of comparing the genetic profile of the studied population groups and to see if there is a population substructure among different regions in Vietnam for this set of markers.

Preliminary results show a high diversity in all samples, except in Lao Cai. In a total sample of 59 unrelated individuals from Lao Cai, 36 have unique haplotypes; and those remaining are shared among 2 to 4 individuals. In the other district groups, a single case of shared haplotypes was found for two samples from Cao Bang (n=41). No shared haplotypes were detected among samples from different districts. In the overall Vietnamese sample, haplotype diversity was 0.9986 ± 0.0009, with an average value of gene diversity over loci of 0.632 ± 0.321.

When comparing samples from different regions, significant differences were observed between Lao Cai and other samples from Vietnam. Nevertheless, no statistically significant differences were detected between the remaining samples.

Until now, the results showed low heterogeneity among distant districts in Vietnam, demonstrating that a geographic criterion is not the most adequate for the structure definition of a forensic database. Meanwhile, it is important to consider that many different ethnic groups exist in the country, which can lead to a significant differentiation of some neighbor districts.

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P24

Application of Rapid Mutating Y-STR markers in forensic cases

Rapid Mutating Y-STRs (RM Y-STRs) have recently been added to the toolbox of the forensic genetic casework scientist (Ballantyne et al 2010 and 2012). RM Y-STRs can be applied in relevant forensic cases that present insufficient resolution with the slower mutating Y-STRs (e.g. from the Y-filer kit). We redesigned the RM Y-STR multiplexes to befit two amplification reactions and validated these for forensic use and application to casework. We present data from criminal cases where RM Y-STRs were applied involving the following questions:

1. Two seemingly unrelated males with matching Y-filer profiles: Can RM Y-STR analysis differentiate these males?

2. A case having multiple related males sharing the exact same Y-filer profile: Can RM Y-STR analysis differentiate these males?

3. A mixture of paternally-related male donors: Which relatives can be excluded or included?

4. Analysis of a very large pedigree: Can RM Y-STRs indicate which branch of the pedigree is more likely to harbour the perpetrator?

5. Cases with (common) Y-filer profiles: Can RM Y-STRs add to increasing the confidence that the true male lineage of a perpetrator is being investigated?

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P25

Familial Searching Combining Autosomal and Y chromosomal STRs and Surnames

POSTER

The introduction of new legislation in the Netherlands in 2012 allows us to conduct familial searching in the DNA-database and in DNA-dragnets. This concept was used in an attempt to identify a relative of the perpetrator of the rape and murder of a young Dutch woman in 1999 in a rural community. We have followed four lines of investigation to identify a (male) relative of the perpetrator:

1. Autosomal STR based Familial search in the Dutch DNA database.

The autosomal DNA profile of the perpetrator was compared to autosomal DNA profiles of 140,000 suspects/convicts using CODIS7 and Bonaparte software. Likelihood ratios (PI and SI) were calculated.

2. A group of approximately 420 males with birthplace or place of residency in the area of the committed crime was selected from the Dutch DNA database. The Y STR DNA profile of the perpetrator was compared to Y STR profiles observed in this group.

3. A group of approximately 260 males that did not live in the direct area of the committed crime but who had rare surnames that reside in the area was selected from the Dutch DNA database. The Y STR DNA profile of the perpetrator was compared to Y STR profiles observed in this group.

4. Voluntary large scale Y STR based familial search amongst several thousands male individuals in the area.

Results of each of the four lines of investigation will be presented.

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P26 Genetic origin of the Kayah Karen in Northern Thailand: Evidence from bi-parental and paternal markers

The Kayah Karen, one of the hilltribes in Northern Thailand, was historically originated from Tibet and Northwestern China. Since a limited number of marker systems in studying genetic structure of the Karen had been reported, current study analyzed an array of 15 autosomal STRs and Y chromosomal haplogroups defined by 80 binary markers from the Kayah Karen (22 males and 22 females) residing Northern Thailand. To address question concerning genetic origin and population affinity of the Kayah Karen, genotyping data of autosomale STRs data of published populations were employed to compare genetic relationship by distance-based and model-based clustering methods. Moreover, Y haplogroup composition was determined to reveal their paternal genetic origin. Autosomal STRs result indicated the closely genetic relationship of the Kayah Karen to various populations from Southern China and Northern Thailand, and genetic admixture in the Kayah Karen. In paternal lineage, six haplogoups, i.e., O2, O3, NO, D1, N, and CT were found in 8, 2, 5, 3, 2, and 2 males, respectively. Haplogroup result indicated an admixed origin of Karen males lineage composing of northern and northwestern China haplogroups equally to Southern China and Southeast Asian haplogroups. Summarizingly, the Kayah Karen was paternally originated from Northern China and subsequently mixed with the native Tai-Kadai and Sino-Tibetan but not native Austroasiatic populations in Southern China and Northern Thailand.

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P27

Central Asian admixture of paternal lineages among contemporary Hungarians

POSTER

The Carpathian Basin had historically been the final destination for many Nomadic peoples who migrated westwards from Inner and Central Asia towards Europe. Proto-Hungarians (Steppe Magyars) themselves came from the Urals area in the early middle ages. However, present-day Hungarian population is genetically very similar to neighbouring European populations.

In order to detect the paternal genetic contribution from Nomadic Steppe tribes, we tested about 1000 samples from Hungarian-speaking European (Hungarian, Sekler and Csango), Central Asian (Uzbekistan, Kazakhstan) and Inner Asian (Mongolia, Buryats) populations.

With the analysis of median-joining networks of certain haplogroups occurred among Hungarian-speaking European, Altaic-speaking Central and Inner Asian populations, we showed that the possible paternal genetic admixture from these populations among contemporary Hungarian populations ranges between 5-10%. It is lowest among Hungarians from Hungary (5.1%), while higher among archaic Hungarian-speaking populations in Romania, notably Szekler (8.3%) and Csángó (9.5%). However, these results represent only an upper limit; actual Central Asian admixture might be somewhat lower as some of the related lineages might have come from a common third source (e.g. Middle East, Caucasus, and East Slavs). The main haplogroups responsible for the Central Asian admixture among Hungarians are J2*-M172 (xM47, M67, M12); R1a1-Z93; Q-M242 and E-M78.

Our study is also interesting regarding the origin of haplogroup R1a1-M198. The solid presence of European branches like R1a1-M458 and R1a1-Z280 in Uzbekistan may not be wholly attributed to modern-era Russian admixture as our Central Asian sample near-completely lacks other prominent European haplogroups (Hg I, R1b-M412). This opens up the possibility for a Central Asian origin of haplogroup R1a1-M198, which should be verified with extensive testing for M458, Z280 and Z93 subclades in the wider region.

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P28 Rearrangement of the AZF gene of the Y chromosome in the nuclear DNA extracted by male sporadic breast cancer tissue

In male it has been observed a correlation between increased susceptibility to the development of testicular seminoma and genetic alterations encompassing AZF gene. AZF gene maps in the eukaryotic region of the long arm of the Y chromosome and it is constituited by three coding regions called AZFa, AZFb, AZFc. When modified AZF gene is strongly implicated in male azoospermia or serious oligospermia. Genetic regions AZFa, AZFb, AZFc have been studied in the nuclear DNA extracted by germinal cells and surgical sporadic breast cancer sample in a male patient. At present the molecular pathogenesis of sporadic breast cancer is unknown. This study shows no genetic alterations of AZF gene germinal line of the male patient. Differently, the analysis of the nuclear DNA extracted by surgical sporadic breast cancer, has shown the presence of genic rearrangement, consisting in a deletion of the M259 region and an insertion among DAZ1-4 region and DYS222 region. The STS marker M259 mapping in the DDX3Y centromeric region results deleted in the nuclear DNA extracted by surgical sample and rearranged in the YP11.3 genetic region. Contextually we typed nuclear DNA extracted by sporadic neoplastic breast tissue with Y-filer and we didn't find genetic alterations in the STR haplotype examined.

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P29

Can Rapid Mutant Y haplotyping be useful to solve cases of complex kinship testing?

POSTER

Y-STR haplotyping is a method used to detect and differentiate male DNA. Y-STR haplotyping is useful in crime casework when is necessary to detect male DNA from a mixed stain (for example, a vaginal swab of a raped and murdered female victim). This method has also proved useful in reconstructing paternal relationship and giving information on the ethnic origin. The principal weakness of Y-STR haplotyping is that patrilineal relatives of the suspect cannot be excluded as being the donor of the stain. Rapid Mutant Y haplotyping is a promising tool to solve this problem.

Our laboratory has observed, in the last years, an increase in the amount and complexity of difficult parentage testing, the most of them are defective in genetic data of putative father. In these cases, we routinely use softwares for calculate the likelihood ratio in parentage/kinship scenarios. We would like to investigate if Rapid Mutant Y haplotyping is a useful tool to solve cases of complex kinship testing.

We analyzed some cases of complex kinship and defective paternity testing, in which were available genetic data of almost two male subjects, variously related to each other. The statistical evaluation and interpretation of parentage investigation cases were initially performed using Familias and PatCan2 softwares, with variable but often inconclusive results. Then we analyzed Rapid Mutant Y haplotyping to verify if this method could be useful to solve the cases, but the results were sometimes misleading.

These cases provide an opportunity for reflection about the potentiality and limits of Rapid Mutant Y haplotyping.

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P30 Genetic polymorphism of 17 Y-chromosomal STR loci in the Kozha and Tore tribes of Kazakh population

The purpose of this research is to study the genetic heterogeneity of the Kazakh populations at the tribal level. The study was performed on the two historically socially privileged tribes (Tore and Kozha) which are not included in the „Shezhire“ system, organizing most of Kazakh clans and tribes. The remote descendants of the steppe aristocracy (Tore N=25) and steppe clergy (Kozha N=74) were analyzed by 17 Y-chromosomal STR markers (DYS389a, DYS389b, DYS390, DYS456, DYS19, DYS458, DYS437, DYS438, DYS448, GATA_H4, DYS391, DYS392, DYS393, DYS439, DYS635). Haplogroup affiliation of each sample was predicted from the STR profile and confirmed by direct analysis of haplogroup-defining SNPs.

For Tore population we observed 17 different haplotypes 92,6% of which were unique, and for Kozha there were 52 different haplotypes 82,7% of which were unique. The haplotype diversity was 0.980 for Kozha and 0.956 for Tore. The variation among populations was FST = 5,61. This level of inter-tribal genetic differentiation is higher than that at the inter-ethnic group level for closely related ethnic groups that have been shown in the literature. The obtained results confirm that, although the Y-chromosomal landscape of one tribe seems fairly homogeneous, genetic heterogeneity is present among tribes.

In the Tore, eight haplogroups were identified, among which there were three major haplogroups: C3 * - M217 (xM48), R1a * - M198 (xM458) and R2a - M124. In the Kozha, there were 14 Y-chromosome haplogroups, only three of which had frequencies higher than 10 percent: R1a1a-M198, J2-M172, R2a-M124. Similarities in spectra of major haplogroups can indicate the genealogical links between the both socially privileged tribes.

This study provides an essential precondition for identification of male DNA and tracing paternal lineages for each tribe.

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P31

Amelogenin test abnormalities in men samples revealed during forensic DNA study and their interpretation.

POSTER

Study of gender markers is a part of routine forensic genetic examination of crime scene and reference samples. Amelogenin gene markers are included in majority of forensic STR kits of different manufacturers. Revealing of sex chromosomes abnormalities in forensic casework samples is rather rare event, mostly there are male samples with no amelogenin Y or X amplification products.

We have found about 100 cases of absence of AMELY amplification product described in scientific literature. We have got 7 cases of AMELY amplification product absence (including two cases of 46,XX male syndrome) and 3 cases of AMELX amplification product absence in men samples during 15 year practice of forensic DNA laboratory in Belarus. Nine of these cases were from crime casework and one was father-son pair in paternity case.

In current study we try to find out probable molecular mechanisms of Y-chromosome abnormalities such as deletions, inversions and translocations with the use of standard kit of forensic laboratory reagents.

Although observed abnormalities are very rare, forensic laboratories should be equipped with at least commercially available kits for Y- and X-chromosome profiling to clarify such cases. Introducing special kits like GenderPlex suggested by Esteve at al. may be useful. Nevertheless, to our opinion usage of Identifiler Plus or GlobalFiler kits for STR loci, Quantifiler Y for SRY gene, Y-filer for determining of possible deletion event and X-chromosome kit such as Argus X-12 for estimating the number of X chromosomes is sufficient for routine laboratory practice.

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P32 Mitochondrial DNA diversities and phylogenetic relationship of four major ethnic groups and Vedda population in Sri Lanka

Sri Lanka, a Southern Asian Island, known as Ceylon until 1972, is located southwest to the Bay of Bengal in the Indian Ocean. It is residence to different ethnicities, having diverse religions and languages. Genetic variations and gene flow of Sri Lankans are still being studied. A few studies of classical and autosomal markers have provided somewhat contradictory evidence on population relationship of Sri Lankans. Analysis of mitochondrial DNA of different ethnic groups and also the statistical and phylogenetic interpretation will provide genetic evidence for the relationship of maternal lineage of contemporary Sri Lankans. Haplotype classifications, genetic variations and phylogenetic relationship are presented based on the mtDNA hypervariable region I and II of the five ethnic groups in Sri Lanka. A total of 180 maternally unrelated individuals from Sinhalese (N=60), Sri Lankan Tamils (N=30), Muslims (N=30), Malays (N=30) and Vedda population (N=30) were included to this study. During the study major population diversity indices were computed to identify significant genetic differences/similarities within and between ethnic groups. Moreover phylogenetic relationship based on the population pairwise FST values were calculated for combined HVI and HVII sequences. Based on HVI and HVII polymorphisms 138 haplotypes were identified. Majority of the haplotypes were unique. Haplotypes shared between ethnic groups indicated a common maternal lineage of recent origin. When considering the haplotype diversities, these populations showed lower inter-population genetic diversities and lower sequence diversities. Vedda group emerged as a different cluster while Moors and Malays were relatively closer to each other than to Sinhalese and Tamils as clearly shown by the Neighbor-joining and UPGMA trees. Hence the Vedda population of Sri Lanka has a distinct maternal lineage compared to other ethnic groups of contemporary Sri Lanka thus confirming historical views that the Vedda individuals are descendants of early inhabitants of Sri Lanka.

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POSTER

P33 mtDNA control region forensic database in the Romanian population and deep investigation of the most frequent haplotypes

Romanian population is composed of 88.92% Romanians, 6.5% Hungarians, 3.29% Roma and 1.29% other populations (2011 census). From the historical point of view Romanians are an admixture of local and surrounding populations. Romania can be divided in 4 major historical regions, each with its particular populations influence: Moldavia that during the past was the Eastern Europe border in front of Mongol, Tatar and Ottoman invasions; Transylvania, where the Austro-Hungarian Empire had an important influence; Wallachia, whose population is the result of Roman Empire conquests, the Slav migration from the north, and the Turkish south-east influence; and Dobruja, in the past conquered by Greeks, Romans, Tatars, Turks and Slavs.

Previous genetic studies made on Y-STR markers suggest that the Slavic influences were dominant and from the perspective of general population (autosomal markers) the dominant influences were Slavic, Italian, Greek and Turkish; unfortunately there are limited data on mtDNA variation in the general population.

In order to analyze the heterogeneity of Romanian population from a mitochondrial lineages point of view and to establish appropriate mtDNA forensic database, we generate a high-quality mtDNA control region data from a Romanian population sample.

400 healthy Romanian donors, from different regions of the country, were subjected to control region sequence analysis. Two PCR fragments were sequenced by using ten different sequencing primers, according to forensic standards. To ensure high data quality at least a double reading of each site and an independent evaluation of electropherograms were performed. A phylogenetic approach for a posteriori analysis of the mtDNA types was applied and sequences were aligned according to the mitochondrial phylogeny.

To increase the utility of mtDNA analysis in forensics, the Personal Genome Machine was used to sequence complete mtGenomes of the most common haplotypes, in order to investigate in more detail specific coding region variations.

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P34 The relation between the genetic mutations of mtDNA and sudden death cases with cardiac hypertrophy

Sudden cardiac death is one of the most common causes of death, especially in young adults. Since the heart has the highest density of mitochondria and relies on brisk oxidative phosphorylation (OXPHOS), mutational analysis of mitochondrial DNA (mtDNA) in myocardial diseases has been widely reported. However, the relation of mtDNA mutations and energetic failure as one of the mechanisms leading to heat failure is still unresolved. Comprehensive screening of whole region of mtDNA was performed in 44 sudden death cases with cardiac hypertrophy (SCDH), in order to evaluate the prevalence of mtDNA mutations in sudden death. DNA was extracted from blood sample of all cases with informed consent of the family members. Mean heart weight was 561g and concentric hypertrophy or dilatation hypertrophy was observed in each case by autopsy. A total of 151 preserved samples that presented with no particular diseases were used as controls. Twenty-four alternations were detected as nonsynonymous mutations only in 17 cases. Six of the mutations were new one. Some mutations were located in highly conserved domains in the genes encoding OXPHOS complexes and/or resulted in a change of the charges of the altered amino acid. Therefore, it was suggested that the mutations detected only in SDCHs in turn affected the ability of essential components of OXPHOS complexes and caused cardiac hypertrophy and failure. In addition, association between mtDNA haplogroups in SDCHs and controls were analyzed. Twelve major mtDNA halogroups in Japanese were identified in this study. Haplogroup G1 was found to have a higher frequency in SDCHs compared to controls (p=0.016). This result indicated that haplogroup G1 are susceptibility factor for developing cardiac hypertrophy.

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P35

Mitochondrial DNA landscape of Tunisia and its position within Mediterranean populations

POSTER

Situated in the Western North of Africa, Tunisia has been occupied by successive civilizations at both prehistoric and historic periods which might contribute to the genetic landscape of the current Tunisian population. With the aims to define the genetic structure of Tunisians and to compare it to other Mediterranean populations, we amplified and sequenced the hypervariable segment of the mitochondrial DNA from 100 Northern Tunisian subjects. Additional Tunisian (715) and Mediterranean mitochondrial DNA sequences (4206) were collected from the literature. Both statistical and phylogenetic analyses were performed.

This study highlighted the mosaic genetic structure of the Tunisian population with the predominance of the Eurasian lineages, followed by the Sub-Saharan and North African lineages. Our results suggested that neither geographical location nor ethnic origins have a major role in the mitochondrial DNA genetic background of the Tunisian population.

Phylogenetic analysis showed that the majority of Tunisian localities were closer to North Africans and Near Eastern populations than to Europeans.

This study provides a global vision of the genetic background of Tunisian and Mediterranean populations which is particularly useful in Forensic and for the design of future studies in several fields such as evolutionary biology, GWAS (Genome Wide Association Study) and pharmacogenomics.

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P36 Mitochondrial DNA data from the Netherlands to improve content and geographic coverage of the EMPOP database

The high-copy-number and maternally inherited mitochondrial DNA (mtDNA) has gained importance as a validated and robust tool in special forensic cases. Hence, the European DNA Profiling group (EDNAP) established an easily accessible high-quality mtDNA population database for forensic purposes (www.empop.org). Such a database is used for frequency estimations of mtDNA sequences observed in forensic casework and hence determine the random match probability, given an exact mtDNA sequence match between the crime-scene sample and a given suspect. Aiming for inclusion in the EMPOP database, we generated the complete mtDNA control-region sequences (16,024-16,569 and 1-576) of over 700 individuals from across the Netherlands sampled at 53 sites covering the entire country with 11 geographic sub-regions. The complete mtDNA control region was amplified with two PCR primers and high-quality sequences were obtained by Sanger sequencing with ten different sequencing primers using the EMPOP protocol. The quality of the mtDNA sequences was checked effectively using quasi-median networks. Phylogenetic analysis of all generated sequences was performed to determine the mtDNA haplogroups. This poster will present a summary of the data obtained and the most important population genetic outcomes, while the data will be made available upon publication in the EMPOP database. Our dataset will improve the overall data content and geographic coverage of the EMPOP database in general, and is particularly aimed to serve as (start of a) national reference database for mtDNA applications in forensic casework in the Netherlands.

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P37

The association of POLG and p53 mutations with mitochondrial genome mutagenesis of colorectal cancer cells

Mitochondrial DNA was found to be highly mutated in colorectal cancer cells. One of the key molecules involved in the maintenance of mitochondrial genome is polymerase gamma (POLG), which plays an essential role in mtDNA replication and repair. Moreover, it was demonstrated that the tumor suppressor molecule, p53, might have a role in maintaining mitochondrial genetic stability through its ability to translocation to mitochondria and interaction with POLG in response to mtDNA damage. Therefore, in the present study we have investigated POLG and p53 sequence variability in colorectal cancer patients for which whole mitochondrial genome sequences were determined. 100 sequences (for tumor and matched non-tumor tissues collected from 50 patients) of POLG gene and exsons 3-9 of p53 were determined. 21 somatic changes in POLG gene were found in 10 colorectal cancer patients, whereas 27 somatic mutations of the analyzed p53 fragment were found in 16 patients. We have found no association between the presence of mtDNA mutations and somatic mutations in POLG or p53 gene. Moreover, no association was found between hereditary mutations in POLG or p53 gene and somatic mutations in mitochondrial genomes. Nevertheless, POLG haplotype analysis demonstrated the presence of "protective" haplotype. This haplotype, without any mutations and intron/splice junction variants, was observed with significantly higher frequency in control subjects.

POSTER

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P38

Mitochondrial Point Heteroplasmy in Human Tissue Structures

For a long time, point heteroplasmy (PHP) has been considered as an uncommon event in mitochondrial DNA (mtDNA), but the development and improvement of new detection methods showed that heteroplasmy is a common phenomenon. Previous studies observed differences between tissues within an individual, but did not look into deeper structures of the tissues. Until now we therefore investigated PHPs in three different skeletal muscles, kidney (renal medulla and cortex), three different brain regions (cerebellum, cerebrum, thalamus), heart (all four cardiac chambers), gastrointestinal tract (stomach, duodenum, jejunum, ileum, appendix, sigma), fat tissue (greater omentum, subcutaneous fat) and blood from 12 individuals. The whole mtDNA control region was analyzed by Sanger-type sequencing and ambiguous PHPs were confirmed by SNaPshot minisequencing and cloning experiments.

First results verified the findings of our study (cf. Abstract: "Frequency and Pattern of Mitochondrial Point Heteroplasmy in Human Tissues") with a high abundance of one or more PHPs in the investigated individuals (11/12). From the newly analyzed tissues, only kidney showed a higher amount of PHPs whereas in gastrointestinal tract and fat tissue lower rates were observed. PHPs at specific positions were seen in kidney, heart and skeletal muscle, but only within the kidney differences between renal medulla and cortex were detected.

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P39

In-solution targeted enrichment of the mitochondrial genome and forensically relevant nuclear SNPs.

POSTER

Routine DNA profiling techniques are generally limited to standard nuclear or Y chromosome STR analysis or to sequencing the control region of the mitochondrial genome. But as more and more informative tests become available, such as SNP typing for inferring ancestry or phenotyping, the analyst can be confronted with a dilemma on which tests to prioritise as forensic samples are generally limited in DNA material. With the advent of next generation sequencing, continuously new library preparation method development and target enrichment, it is now possible to combine a high number of tests into one single custom assay.

A commonly used method, in-solution targeted enrichment, originally developed for ancient DNA analysis, was adapted and optimised for forensic use in a routine laboratory setting. Fragmented and indexed mitochondrial genome target was hybridised to complementary biotinylated bait produced by long-range PCR and adapter ligation. Streptavidin coated magnetic beads and a strand-displacement enzyme were subsequently used to isolate and enrich the target sequences for sequencing on the MiSeq platform.

We have applied this technique to recover the entire mitochondrial genome and are currently expanding the assay to encompass forensically relevant nuclear SNPs. Immortalising both bait and target fragments through low cycle PCR allows limitless stock to be available for retesting if and when needed. Moreover the utilisation of indexing, the addition of a small unique sequence to the ends of target fragments, allows for the multiplexing and capture of DNA from several samples at once, reducing time and cost.

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P40 Genetic portrait of Lisboa immigrant population from Angola with mitochondrial DNA (preliminary results)

Portugal has been considered a country of emigrants, nevertheless in the past decades the number of immigrants has grown through all the country. This migratory flux has contributed to a raise of heterogeneity at multiple levels. According to statistical data, until the end of 2012 total number of Angolan immigrants in Portugal equalled about 20.000 individuals. A territorial predominance has been found for the metropolitan region of Lisboa.

The presence of Bantu people and the colonization by Portuguese people on Angolan territory are considered to be the major modulators of genetic patterns in this country.

Maternal heritage of mitochondrial DNA (mtDNA) allows us to determine the maternal ancestry of many generations.

Blood samples from 88 Angolan immigrants residents in Lisbon, which attended Delegação do Sul do INMLCF for kinship investigations, were collected. DNA was extracted using Chelex®100 method. Control region of the mtDNA was amplified using two pairs of primers – L15997/H016 and L16555/H599. The amplified products were purified, followed by sequencing with BigDye® Terminator v.3.1 Cycle Sequence (AB). Sequenced products were detected in a sequencer Genetic Analyzer 3130 (AB) and the results were analysed by Sequencing Analysis v.5.2 and SeqScape v.3 (AB). The sequenced segments were compared with revised Cambridge Reference Sequence (rCRS) applying the nomenclature guidelines used in mtDNA analysis. Haplogroups were determined based on Phylotree, build 15.

In this study, 72 unique haplotypes were identified and 8 were shared within groups of 2 individuals. Out of 88 haplogroups, 77 belong to macrohaplogroup L; 3 to haplogroup R, 2 to haplogroup K and 2 to haplogroup U. The haplogroup H, J, M and T were present only one time. 87,5% of this results show haplotypes characteristic of the subshariana region, where Angola is located. This study demonstrated the genetic diversity that this immigrant population particularly introduces in Lisboa region.

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P41

A new protocol to enable the analysis of inhibited mitochondrial DNA samples.

The analysis of mitochondrial DNA (mtDNA) has become an important part of the analysis of the probative value in forensic casework. At the Netherlands Forensic Institute two mtDNA approaches are used: standard mtDNA typing (id est 3 medium-sized amplicons for HVS1, HVS2 and HVS3) for reference samples and mini-mtDNA typing (id est 10 small-sized amplicons in 2 multiplexes [1]) for compromised samples. Often the samples are of low quality and/or low quantity, which make them especially prone to inhibitory effects of possible contaminants. To tackle this issue, we tested the Qiagen Multiplex PCR buffer in stead of the PCR components provided with the AmpliTaq polymerase. Several inhibitors (hematin, tannic acid and melanin) were added in various concentrations to the mini-mtDNA amplifications. While no PCR products and mtDNA sequences were obtained with the AmpliTaq reagents, all specimens resulted high amounts of PCR product and correct sequence reads when the Qiagen Multiplex PCR buffer was used. Our results demonstrate that the use of this PCR buffer allows for an easy and robust protocol, useful for the forensic community working on forensic mtDNA casework.

[1] Berger and Parson, Forensic Science International: Genetics, volume 3, 2009, 149-153

POSTER

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P42 POLYMORPHISM OF THE MITOCHONDRIAL DNA CONTROL REGION IN THE POPULATION OF SERBIA

Our study was aimed to improve the knowledge of existing mtDNA variations in contemporary population of Serbia and to expand the existing database, since data regarding HVS-I and HVS-II sequence polymorphisms for population of Serbia is limited. Therefore, we have analyzed sequence polymorphisms of the HVS-I and HVS-II of the mtDNA control region in combination with screening of coding region haplogroup-specific RFLP markers in 100 individuals from the general population of Serbia. We have identified 87 different haplotypes resulting from 103 polymorphic positions. The most frequent haplotype in our population sample (263G 315.1C) is also one of the most common haplotypes in other European populations. The genetic diversity was found to be 0.9966. The probability of two random individuals showing identical mtDNA haplotypes was 1.3%. We observed that transitions made up the majority of variations (72.10%) while transversions were less represented (1.76%). The majority of the mtDNAs observed in our study belong to the most common European haplogroups. In addition, frequency distribution of the mtDNA haplogroups in population of Serbia is found to be similar to that found in other European populations.

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P43 Full mtGenome reference population data: Development and evaluation of 588 forensic-quality haplotypes

POSTER

As part of a U.S. National Institute of Justice funded project, we have developed 588 complete mitochondrial genome (mtGenome) haplotypes, spanning three U.S. population groups, from anonymized, randomly sampled blood serum specimens. First pass data generation (PCR followed by Sanger sequencing) performed in semi-automated fashion using robotic instrumentation was highly successful despite the typically very low DNA quantity of the sample extracts; and a carefully considered strategy for targeted sample reprocessing in cases of PCR or sequencing failure minimized the extent of manual sample handling. Data production metrics developed from the laboratory processing effort demonstrate that a) large (approximately 2.5 kilobase) mtDNA fragments can be routinely recovered from low template specimens, and b) forensic-quality complete mtGenome haplotypes can be generated in a production-type (i.e. casework-like) environment. Data review followed a workflow previously used to generate high-quality mtDNA control region (CR) data sets, and included multiple reviews of the raw data, and comparison and correction of the independently-generated haplotypes to arrive at 100% final concordance. As an additional quality control measure given the use of a multi-amplicon PCR strategy, mtGenome haplotypes were compared to the mtDNA phylogeny to confirm that each haplotype represented a single sample.

We will present our data production and review workflows, and the processing metrics developed from the low template samples employed for this databasing initiative. Additionally, we will present detailed analyses of the completed data sets, including summary statistics for the full mtGenome in comparison to subsets of the molecule (e.g. the hypervariable regions and the CR) as well as haplogroup/continental origin frequencies and distributions for each population. Ultimately, this work will provide the forensic community with reliable, complete mtGenome reference population data, and a model for development of additional mtGenome data sets using both new and currently validated technologies.

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P44 Resolving the most common West Eurasian mtDNA control region haplotype in an Italian population sample by massively parallel mtGnome sequency

The high mutation rate of mitochondrial (mt)DNA, its lack of recombination, its high abundance in the cell and its greater resistance to environmental stress make the analyses of this molecule the most promising choice in forensic genetics when nuclear markers fail to give reliable results. The control region (CR) shows the highest variability and is therefore routinely typed in forensic studies. Coding region data are currently used almost exclusively for a phylogenetic assignment of mtDNA haplotypes and in population genetic studies. The extended analysis of the mitochondrial genome covering also the coding region is however also useful in forensic applications, e.g. to further differentiate identical CR haplotypes.

The most frequent western Eurasian mtDNA CR haplotype 16519C-263G-315.1C (with respect to the rCRS) has been observed in various sub-haplogroups of haplogroup R0 (Brandstätter et al., 2008). In the current study 29 samples that displayed this haplotype were selected from a larger Italian population study (Boattini et al., 2013) and sequenced for their coding region by massively parallel sequencing as previously described (Parson et al., 2013).

The obtained complete mtGenome haplotypes were assigned to their established sub-haplogroups and interpreted with respect to their phylogenetic and geographic background. This study clearly demonstrates the benefit of full mtGenomes to increase the resolution of mtDNA sequencing in forensic genetics.

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POSTER

P45 Sequence polymorphism of the mitochondrial DNA hypervariable regions I and II in the population of Vojvodina Province, Serbia

In order to generate and establish the database for forensic identification purposes in Vojvodina Province (Serbia), the sequence of the hypervariable regions 1 (HV1) and 2 (HV2) of the mtDNA control region were determined in a population of 106 unrelated individuals from Vojvodina Province, using a fluorescent-based capillary electrophoresis sequencing method. A total of 95 different haplotypes were found, of these 85 mtDNA types were unique, 9 haplotypes were shared by two individuals and 1 haplotype by three individuals. The variation of mtDNA HV1 and HV2 regions was confined to 116 nucleotide positions, of which 72 were observed in the HV1 and 44 in the HV2. The majority of mutations in both regions were transitions 75.98%, insertions were observed in 21.44%, transversions in 2.44%, and deletions in 0.13%. A statistical estimate of the results for this population showed the genetic diversity of 0.9978 and the random match probability of 1.16%. All nine common European haplogroups (H, I, J, K, T, U, V, W, and X) were observed in the sample. The most common haplogroup in Vojvodina was H (43.4%), as is common in Europe. Haplogroups observed at intermediate levels included clusters U (13.2%), T (10.4%), J (8.5%), W (5.7%) and V (4.7%). The haplogroups observed less frequently include K, I, X, B4, N1a and N1b and these were observed in approximately 3.7% or less of the sample.

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P46

Using DNA barcodes to identify forensic species of Diptera in Southeast Brazil

Forensic Entomology is the study of insects, mites and other arthropods of forensic importance, in order to solve cases and court procedures. Success in research and related work in this area depend solely on a correct identification of those animals. However, an accurate identification of insect species is not an easy task to accomplish and researchers have resorted to identification keys or the contribution of taxonomic experts, mainly for Diptera Order. Due to problems with the identification of animal species based only on their morphology, new methods have been developed, including species identification by DNA. One such method corresponds to the DNA barcode, which uses universal primers to amplify a specific region of mitochondrial DNA for species identification. Thus, the objective of this study was to identify forensically important species of Diptera in Southeast Brazil using DNA barcode. For this propose, samples were collected in four different locations of our region using traps made from PET bottles, containing ground beef for the attraction of flies. Morphological identification was made using identification keys. DNA sample was extracted with Chelex resin and the mitochondrial DNA gene Cytochrome c Oxidase subunit I (COI) was amplified by PCR, using universal primers, and sequenced. Sequencing results were compared with BOLD and GenBank databases to confirm the morphological identification. As result, 281 adults were collected and approximately 10% of those samples were analyzes by DNA. It was possible to identify Atherigona orientalis, Musca domestica, Chrysomya megacephala and one species of Sarcophagidae (Oxysarcodexia thornax). Some samples could not be identified because they showed less than 97% of similarity in databases and also some samples were identified as Bos taurus (the substrate for attracting flies) showing the problem of unspecific amplification using universal primers in forensic entomology.

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P47

Coding region SNP analysis to improve dog hair mitochondrial DNA profiling for forensic purposes

POSTER

Despite the frequent encounter of dog hair in casework and its potential as associative evidence, canine mtDNA analysis is still uncommon in forensics. One of the reasons is the canine control region's limited discriminative power. In numerous population studies worldwide, three control region haplogroups were observed in nearly half of the dogs. Clearly, although there are also many rare control region haplotypes, an mtDNA match between a trace and a given dog will frequently have limited forensic value.

So far, only a few studies have been published that explore the entire canine mitochondrial genome to increase its discrimination power. Our own study was focused on the three most common control region haplotypes of a Belgian control region study. The whole mitochondrial genome sequence was assembled for 81 dogs of these three types. A total of 26 forensically informative single nucleotide polymorphisms (SNPs) were identified in the mtDNA coding region. These variable sites resolve the three control region types into 24 groups of mitochondrial genome haplotypes. The groups have estimated population frequencies of 0.5 to 9% based on the initial Belgian dog population study. This is a substantial improvement compared to the approximately 15% frequency of each of these three control region haplotypes. Using three newly designed SNP assays targeting the 26 variable sites, the frequency estimates were also evaluated against a second Belgian dog population study.

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P48

A forensic database of cat mitochondrial DNA variants

In July 2012, the dismembered torso of a local man washed up on a beach in Southsea, England, wrapped in a shower curtain within a sealed plastic garbage bag. Investigators found no trace of the murderer's DNA but a number of cat hairs were recovered. The main suspect possessed a cat of similar colour and DNA testing by the Veterinary Genetics Laboratory (UC Davis) showed a matching mitochondrial DNA profile between this cat and the hairs on the torso's wrappings. In the absence of published information regarding the frequency of the mitotype in the UK, we constructed a database of mitochondrial DNA sequence variation from randomly sampled cats both locally and nationally. Sanger sequencing of 402 bases of the mitochondrial control region from 152 cats showed a preponderance of common sequences detected in other global population studies and rarer variants so far only observed in the UK.

The rarity of the profile in this case provided an additional strand of evidence linking suspect and victim. Work is ongoing to expand the coverage of the database and improves its discrimination so that forensic DNA testing of cat hairs can be applied more widely.

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P49

Performance of the AutoMate Express for DNA extraction from old skeletal remains

In forensic genetics investigations old skeletal remains are among the most challenging biological samples for successful STR typing. For genetic identification of skeletons it is necessary to obtain sufficient high-quality DNA. In this study we tested the performance of AutoMate Express (AB) for DNA extraction from old skeletal remains. We used femurs, tibias and teeth (molars) from 26 skeletons excavated from two WWII mass graves in Slovenia. STR typing of autosomal DNA was performed to evaluate the performance of tested extraction method. We cleaned the bones and teeth, removed surface contamination, and ground them into powder using liquid nitrogen. Prior to DNA extraction using PrepFiler Express BTATM Forensic DNA Extraction Kit (AB) 0.5 g of bone or tooth powder was decalcified. The nuclear DNA of the samples was quantified using the QuantifilerTM Human DNA Quantification Kit (AB). We extracted up to 15 ng DNA/g of tooth powder, up to 6 ng DNA/g of femur bone powder, and up to 4 ng DNA/g of tibia bone powder. STR typing was performed using the AmpFlSTR NGMTM PCR Amplification Kit (AB). We obtained almost complete genetic profiles from all skeletons except one. For traceability in the event of contamination, we created an elimination database including genetic profiles of all persons that had been in contact with the skeletal remains and no match was found. Extraction of DNA using AutoMate Express (AB) has proven highly successful in the recovery of high-quality DNA from bones and teeth from WWII victim's skeletal remains. We believe that the results obtained could contribute to the possibilities of the use of automated devices for extracting DNA from old skeletal remains, which would shorten the procedures for obtaining high-quality DNA from skeletons in forensic laboratories.

POSTER

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P50

Serial killers identified with DNA

Serial murders occurred in the last 6 years in two different cities in the Republic of Macedonia. Four males were killed on a similar way in the city of Ohrid and only females, same age, in the city of Kicevo. All of the bodies were in a process of decomposition or were skeletonized. Autopsy was performed on all victims, and DNA from bones of the victims, vaginal swabs, fingernail debris and biological traces on clothes were analyzed. Extraction of bones was performed by using of phenol-chlorophorm method, and out of the other materials we used Qiagen Micro kit. For amplification were used Indentifiler ABI, Y-filer ABI and Power Plex 16 Promega kits. Post amplification products were analyzed by using 310 Genetic Analyzer ABI. DNA View program was used for making statistical analyzes. One skeletonized body of the victims was identified in the city of Ohrid and the mechanism of injuries on his body was proved. In the second case, in the city of Kicevo, in the vaginal swabs of all female victims, DNA of the perpetrator was found. DNA was the only material evidence in those two cases, so the perpetrators of the crimes were arrested.

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P51

Genetic analysis of bone samples - our experience

In many cases the only available material for human identification is a bone sample. Genetic identification based on bone samples is particularly demanding because of the reduced amount of genetic material, degradation induced by environmental exposure and the presence of potential inhibitors. In this work we present our approach validated by the results obtained for 50 cases requiring forensic genetic analysis of DNA extracted from bone tissue. We analyzed different parts of a skeleton: dental elements, long or flat bones. We optimized and applied specific pre- and post-extraction methods, which allowed us to obtain a valid genetic profile in most cases. Genetic analysis was based on STR (short tandem repeats) markers, obtained using validated commercial kits, taking into account the risk of stochastic effects increased in such difficult cases.

The acquired experience and the results obtained with different protocols indicate the importance of the bones' origin or age, as well as the necessity for a thorough preparation and rigorous interpretation of results.

POSTER

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P52 The identification of Edward 'Ned' Kelly remains, some 130 years after his burial, and the role of mitochondrial DNA analysis

Edward (Ned) Kelly was a notorious Australian outlaw ('bushranger'), regarded by some as charismatic and by others as one of Australia's cold blooded police killers. Ned Kelly was executed in 1880 and buried at the former Melbourne Goal in Victoria, Australia. In 1929, the remains of executed prisoners were disinterred from the Melbourne Goal and reburied at the Pentridge Prison. The Pentridge Prison site was subsequently sold for commercial development in 1999, leading to the excavation of three burial pits between 2008 and 2009 (which were thought to include the remains of Ned Kelly). In 2010, human skeletal remains recovered from the burial pits were brought to the Victorian Institute of Forensic Medicine (VIFM) for curation and analysis.

A specialized team of VIFM staff and colleagues from the Argentine Forensic Anthropology Team (EAAF) was established to address the question of what happened to Ned Kelly's remains. The identification effort consisted of a multidisciplinary approach including scientific disciplines (forensic anthropologists, pathologists, odontologists, and geneticists) as well as historians and legal experts. Given the antiquity and condition of remains recovered from the Pentridge site, and the 130 years that had passed since Ned Kelly's execution, mitochondrial DNA analysis was chosen as a suitable DNA analysis tool to examine the Pentridge cases to assist in the inclusion or exclusion of remains as being those of Ned Kelly. Only one set of human remains matched the HV1/HV2 mitochondrial DNA haplotype of a maternal descendent of Ned Kelly. Additional anthropological analyses indicated a number of pathological features that provided support that these remains were those of Ned Kelly. This paper reports on the DNA evidence which supports the identification of Ned Kelly.

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P53

Estimation of homozygote/heterozygote drop-out probabilities and internal validation of a mixture profiling method

POSTER

Allelic drop-out probabilities were estimated using logistic analysis (Gill et al. 2012) performed on DNA profiles of our real crime scene samples. Altogether 281 single source, low level (≤1000RFU) NGM SElect and PowerPlex ESI 17 DNA profiles were analyzed. All investigated profiles had at least one allelic drop-out as compared to the reference samples. Profiles amplified with normal (29-30x) or increased (+2) cycle numbers were handled separately. The logit plots of the homozygote and heterozygote drop-out probabilities were different between the two kits: lower drop-out probabilities were estimated from NGMS SElect profiles. In addition, homozygote and heterozygote drop-out probabilities were calculated for three locus size ranges. As expected, the drop-out probabilities increased with the fragment size.

For our internal validation of forensic DNA profiling protocols we also tested an automated composite-consensus method (Bekaert et al. 2012) which separates major consensus alleles from minor or non-consensus ones by a system of brackets. This method generates DNA profiles from PCR replicates and uses allelic balance threshold (ABT) to identify the correct major donor. We analyzed artificial low (20 pg), medium (100 pg) and high (500 pg) template three-person mixtures with low (1:1.5:3), medium (1:2:5) and high (1:5:10) donor ratios. Two mixture panels were created: one with a dominant male/minor females and the other with a dominant female/minor males. Altogether 18 samples were amplified two and four times with NGM SElect and PowerPlex ESI 17 kits. The optimal ABT values and the ratios of correct dominant profiles were determined for both the STR loci and the Amelogenin locus in the two and the four PCR replicate systems. Our results demonstrates that this composite-consensus method is a useful general tool for automated evaluation of both normal and low level PCR replicates of NGM SElect and PowerPlex ESI 17 systems.

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P54 Population data and forensic parameters of 30 insertion/deletion polymorphisms of Cabo Verde immigrants in Lisbon

In the 15th century, the Portuguese settlers discovered the Cabo Verde islands. Santiago and Fogo islands served as a base for exploratory missions to the West African coast and later, were used as a stop in transatlantic commercial slave smuggling. Slaves were captured between Senegal and Guinea, brought into the islands and sold in South America and Europe. The settlement of the islands involved European males, mainly Portuguese, and because no women were among them, the settlers formed liaisons with slave women, originating the Cabo Verde population. During the past six centuries, the islands suffered several tough periods, which led to mass migrations to Europe, mainly Portugal. Nowadays there are about 42 400 emigrants from Cabo Verde living in Portugal, and from those, 34 000 reside in the Lisbon region.

Considering the history between Portugal and Cabo Verde, and the actual number of immigrants living in Lisbon, our aim was to characterize the population of Cabo Verde immigrants with 30 InDel (insertion/deletion polymorphism) and evaluate those genetic markers for forensic purpose. InDels are biallelic length polymorphisms, created by insertion or deletion of a few nucleotides in the genome. These markers have several advantages that make them useful for forensic research.

A total of 403 individuals from Cabo Verde residing in Lisbon, who attended our laboratory for kinship research, were selected for InDel typing. The DNA was isolated from blood stain cells with Chelex 100 method and amplified with commercial kit DIPplex Investigator® (QIAGEN).

Population data, as allelic frequencies, observed and expected heterozygosities and Hardy-Weinberg equilibrium were calculated with Arlequin software, v.3.5. The forensic parameters such as discrimination power, power of exclusion, polymorphic information content, paternity index and match probability were calculated using PowerStats software, v.12.

The data obtained is compatible and appropriate for forensic purpose.

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P55

Genome-wide methylation profiling and a multiplex construction for the body identification using epigenetic markers

The identification of body fluids found at crime scenes can contribute to solving crimes by providing information that reveals important insights into crime scene reconstruction. In the present study, a multiplex methylation SNaPshot system was developed for forensic body fluid identification using body fluid-specific epigenetic markers, which were identified from genome-wide epigenetic analysis of 24 body fluid samples using the Illumina Infinium HumanMethylation450 BeadChip array. A total of 66 CpG sites were found to have more than 30% discrepancy in DNA methylation status between a certain type of body fluid and other types of body fluids, and to have body fluid-specific complete methylation or unmethylation pattern. From further locus-specific methylation analysis in additional samples, one to three CpG sites were selected for each body fluid. Then, multiplex methylation SNaPshot reaction was constructed for the analysis of methylation status of 8 body fluid-specific CpG sites. The developed multiplex can identify blood, saliva, semen, and vaginal fluid in one reaction and also produced successful DNA methylation profiles in aged or mixed samples. Although it remains to be investigated whether this approach is more sensitive and more practical than RNA- or peptide-based assays, the newly developed multiplex method ensures forensic applicability as well as high specificity and reliability, thereby facilitating more efficient body fluid identification in forensic casework.

POSTER

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P56 Integrated Forensic DNA Data Analysis and Management - A scalable enterprise solution for forensic DNA laboratories

Management and analysis of DNA workflow data in forensic labs is a daunting task. One of the most challenging aspects is the ability to easily and efficiently extract useful intelligence out of related, but currently disjointed sets of data stored across a variety of systems within the laboratory. With NGS technologies, additional information gathered from Single Nucleotide Polymorphisms (SNPs), Y chromosome Short Tandem Repeat (Y-STR), and mitochondrial DNA (mtDNA) also become factors to be considered simultaneously to further enhance the intelligence.

Life Technologies is developing a new enterprise software solution suite that seamlessly integrates volumes of raw, processed and meta data generated throughout the DNA processing workflow, from sample accessioning, extraction, quantification, amplification to CE and fragment analysis. The software solution is highly configurable to fit specific laboratory workflows, and SOP. The software allows for automated data transfer with various forensic DNA laboratory instrumentation including Life Technologies' real-time PCR and CE instrumentation, as well as, its GeneMapper® ID-X analysis software. The software supports a scalable local DNA profile database with the capability to store autosomal STR, Y-STR, mtDNA, and SNP profiles. And can be searched for single source profiles complete and partial matches using a standard Identity By State (IBS) method, and relatives with a novel familial search algorithm developed by Life Technologies. This data management capability can be extended to further build Y-STR Haplotype Frequency databases intrinsic to particular region for casework. The system also allows for configuration of the user interface and output reports, as well as features to insure data security and integrity. The characteristics, configurability, and analytical search capabilities of the software will be presented.

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P57

Development Process Validation for Kinship Analysis Algorithm

Product

> Kinship and Paternity Analysis module will be called - Converge(TM) Kinship and Paternity

> Local database direct profile match module will be called - Converge(TM) Direct Search

> Local database familial profile match module will be called - Converge(TM) Familial Search

Computing likelihood ratio in kinship analysis for autosomal markers is straightforward and well defined. Such calculation provides a value for evidence given the prosecution versus the defense proposition. It is recommended and widely used in forensics, missing person and paternity. The forensics community has validated stand-alone software for calculating LR using trios and many biologically related family members. Software such as Familias and MPKin are used regularly for such calculations. Because these implementations are standalone, transcription errors can occur on transferring data from data collection, table input and result storage, in addition it can also be time consuming. Life Technologies has incorporated its version of a kinship algorithm to data collection and storage for ease of use and reliability of results, therefore avoiding human transcription errors. This presentation will encompass the steps taken by our team to validate our kinship analysis algorithm given the available methods, data and external collaborators. Building on previous literature, we have used NIST, CEPH and real data from collaborators to compare results of the Life Technologies kinship algorithm to those currently used in the paternity and forensics laboratories. We will show that the standard calculations, including complex pedigree trees, mutations and rare alleles concur with currently used methods. We hope to establish this kinship analysis algorithm and be able to use it for NGS data, once expert data and tables become available.

POSTER

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P58

Stability of DNA stored in EO treated environment

Most consumables for forensic DNA analysis, such as tubes, pipette tips or swabs, undergo treatment with ethylene oxide (EO) to remove possible DNA contaminations prior to using them on forensically relevant biological materials.

EO is a very small molecule with good penetration characteristics. It is highly reactive and a potent DNA decontaminant. Originally, the EO technique has been developed to remove biologically hazardous contamination from medical instruments and devices. Despite being a powerful decontamination procedure, the technique has recently been replaced by alternative sterilisation techniques because residual EO may constitute a serious health risk for patients. EO causes spontaneous mutations and profound DNA damage.

The aim of the current study was to test whether or not traces of EO causes further DNA damage during storage of (extracted) DNA in materials which have been treated with EO prior to usage.

DNA has been transferred onto swabs and into tubes and left under routine storage conditions. Samples were analysed after different time points to reveal possible loss of high molecular weight DNA during storage.

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P59

Expanding X-chromosomal forensic haplotype frequencies database: Italian population data of four linkage groups

Requests for solving complex kinship casework involving at least one female are increasing in our experience and in these circumstances the analysis of X-chromosomal STR markers plays a relevant role. Actually, it is well known the superior statistical power of X-STRs compared to autosomal markers in solving relationship when two sisters or half-sisters are involved and none of parents is available, in maternity testing or in cases involving close relatives as alternative putative fathers. In addition, the possibility to amplify more loci simultaneously and the strategy based on the analysis of four linkage groups to obtain the X-haplotype provide a powerful and validated tool. Nevertheless, haplotypes frequency distribution in different population is still needed for calculation of probabilities in relationship testing. Generally, for calculation we use published haplotype frequencies from German population data, but in different caseworks we found unreported X-haplotypes. The recent collaborative multi-center study on 12 forensic X-STR loci highlighted that the most important challenge is to derive sufficiently accurate haplotype frequency estimates for the four linkage groups in different world populations for correct likelihood calculation in kinship testing and proposed that scientists share their data with the forensic genetics community in the dedicated X-chromosome website. To enlarge the forensic X-chromosome database, we present haplotype frequencies and other parameter of forensic interest obtained from 200 anonymous DNA samples of unrelated Italian males for the four linkage groups included in the Investigator Argus X-12 kit. Finally, casework examples of complex kinship analysis are presented.

POSTER

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P60

DNAscan™ Rapid DNA Analysis™ System: Presentation of Data
«DNAscan™ Rapid DNA Analysis™ System: Presentation of Data

Introduction: The DNAscan System is a fully integrated, automated system for the generation of short tandem repeat (STR) profiles from buccal swab samples for forensic and law enforcement use. The system consists of a fully integrated instrument, a single-use consumable with on-board room temperature stable reagents, and expert system software. When incorporated into forensic laboratory or police station workflow, the DNAscan System will increase the speed of DNA processing and help lower the cost to law enforcement by providing a rapid biometric link between a criminal activity and an individual being held in custody.

Materials and methods: A set of experiments was conducted to test the fully integrated, fully automated generation of STR profiles. The performance verification studies were designed and conducted to evaluate the "swab in to profile out" system as a whole, and the expert system software as an individual component. The primary studies included first pass success rate, sensitivity, reproducibility, concordance, and precision.

Results and discussion: The results of the performance verification testing on fresh and dried buccal swabs will be presented and demonstrate that the system is appropriate for fully automated generation of STR profiles. The DNAscan System has been demonstrated to show precision of less than 0.15 SD with single base resolution and 100% concordance. The results of the instrument performance testing 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.

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P61

POWERQUANT™ SYSTEM: A NEW ROBUST HUMAN AND MALE SPECIFIC DNA QUANTIFICATION SYSTEM THAT MONITORS DNA INTEGRITY

POSTER

Current qPCR based quantification systems are highly sensitive and provide accurate human (autosomal) and male (Y) quant values for optimal input of human DNA to add to an STR amplification reaction. The autosomal/Y ratio also assists in determining whether autosomal or Y-STRs assays are likely to be more appropriate. While the quantification values provided by currently available kits are sufficient for downstream processing of single source samples, casework samples present additional challenges such as limited quantities of DNA and poor quality DNA.(Moreover, the new generation of STR kits are extremely robust and perform extremely well in the presence of inhibitors. Therefore, for a quantification kit to provide usable data for downstream processing, it must be sensitive, reliable, and comparable in inhibitor tolerance to the latest generation of STR kits and should provide information on DNA integrity. To address this need, Promega has developed a new robust quantification system which is highly sensitive and which incorporates a new larger autosomal amplicon that may be used to monitor the integrity of a DNA sample. This larger amplicon is generated from a separate region of the same multi-copy autosomal target used for quantification, and thus allows for determination of DNA degradation even at very low template.

We will present data demonstrating sensitivity, resistance to inhibitors, ability to detect DNA degradation, species specificity, and male specificity at various ratios of male to female DNA.

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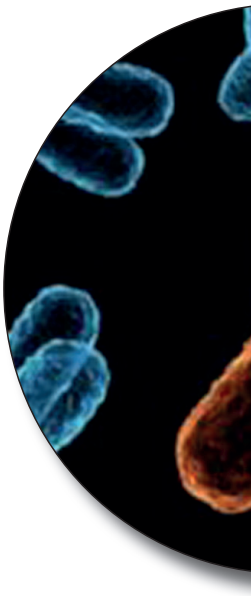

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