DNA IN FORENSICS 2012

"EXPLORING THE PHYLOGENIES"

5th EMPOP Meeting
8th Y-Chromosomal User Workshop

Innsbruck, Sep 06-08 2012

Abstract book &
Conference program

Dear friends, colleagues and guests,

The fields of mitochondrial (mt)DNA and Y chromosome analysis enjoy fruitful development as witnessed by the growing scientific contributions in different genetic disciplines. An important basis for guiding research and exchange of ideas are international meetings.

We welcome you to **DNA in Forensics 2012** in Innsbruck. The motto of this meeting is **Exploring the Phylogenies**. Haploid markers are inherited along a phylogeny and therefore left genetic footprints in modern populations. The incisiveness of the picture depends on the history of populations and their migration. Forensic scientists are taking advantage of the derived patterns in their daily work, e.g. by assigning geographical landscapes to lineages or by performing quality control of datasets. We have received very interesting contributions from the forensic, population and medical genetic fields and look forward to their presentations and vivid discussions.

We wish all participants some beautiful days in Innsbruck!

Sincerely,

Walther Parson and Lutz Roewer

We gratefully acknowledge our Industry partners – Life Technologies / Applied Biosystems, Promega, QIAGEN, Abbott, Qualitype - and Sponsors – Unilab Laboratory Equipment, Bartelt GmbH, Biotype Diagnostics and Fischerlehner + Kutschera Handelsgesellschaft m.b.H.

AGENDA

Thursday, Sep 06

10h00 - 14h00 **Registration**

14h00 - 14h30 **Welcome**

Richard Scheithauer (Innsbruck, Austria)

Mechthild Prinz (New York, USA)

Lutz Roewer (Berlin, Germany)

Walther Parson (Innsbruck, Austria)

Session I: Forensic aspects of the mitochondrial phylogeny
Chair person: Walther Parson

14h30 - 15h00 **Haplogrouping and phylogenetic analysis of mtDNA profiles**
Hans-Jürgen Bandelt (Hamburg, Germany)

15h00 - 15h30 **A ‘‘Copernican’’ reassessment of the human mitochondrial DNA tree from its root**
Doron M Behar, van Oven M, Rosset S Metspalu M Loogväli E-L, Silva NM, Kivisild T, Torroni A, Villems R (Haifa, Israel)

15h30 - 15h45 **PhyloTree - growing and refining the human mitochondrial DNA tree**
Mannis van Oven (Rotterdam, Netherlands)

15h45 - 16h00 **The EMPOP concept for reporting mtDNA haplogroups in forensic genetics**
Alexander W Röck, Dür A, Parson W (Innsbruck, Austria)

16h00 - 16h15 **Improved visibility of character conflicts in quasi-median networks**
Bettina Zimmermann, Hiltpolt B, Röck AW, Dür A, Parson W (Innsbruck, Austria)

16h15 - 16h30 **Evaluating mtDNA profiles: Even the smallest difference has a size**
Stijn Desmyter, Parson W (Brussels, Belgium)

16h30 - 17h00 **Coffee break**

Session II: Applications of mtDNA analysis
Chair person: Lourdes Prieto

17h00 - 17h15 **Linguistic isolates in Portugal: insights from the mitochondrial DNA pattern**
Quim Mairal, Santos C, Prata M, Aluja M, Alvarez L (Barcelona, Spain)

17h15 -17h30 **Genetic microdifferentiation patterns in the mitochondrial DNA pool of Asturias (Northern Spain)**
Antonio F. Pardinas, Garcia-Vazquez E, Roca A, Lopez B (Oviedo, Spain)

17h30 - 17h45 **A detailed phylogeny of rare mitochondrial DNA haplogroups from negrito populations of Southeast Asia**
Phillip L. Endicott (Paris, France)

17h45 - 18h00 **Regional sampling connects population genetics with forensics – an example from South America**
Martin Bodner, Perego UA, Huber G, Fendt L, Röck AW,Zimmermann B, Olivieri A, Gómez-Carballa A, Lancioni H, Angerhofer N, Bobillo MC, Corach D, Woodward S, Salas A, Achilli A, Torroni A, Bandelt H-J, Parson W (Innsbruck, Austria)

18h00 - 18h15 **Genetic discovery of early medieval human skeletal remains based on gender and mtDNA data**
Christiane M Bauer, Niederstätter H, Mayr-Eduardoff MA, Berger C, Huber G, Stadler H, Parson (Innsbruck, Austria)

18h15 - 18h30 **Forensic science applied on prehistoric remains - a nine fold burial of the 4th millennium BC raised questions about kinship, locality, and circumstances of death**
Sarah Karimnia, Schlenker B, Stecher M, Bauer CM, Niederstätter H, Parson W, Friederich S, Meller H, Alt K (Mainz, Germany)

18h30 - 18h45 **Different mutational spectra of complete mitochondrial genomes of normal and colorectal cancer cells.**
Katarzyna Skonieczna, Malyarchuk B, Jawien A, Marszalek A, Grzybowski T (Bydgoszcz, Poland)

18h45 - 19h00 **MtDNA haplogroups and sudden infant death syndrome**
Stephan Köhnemann, Schumann S, GeSID group,
Pfeiffer H (Münster, Germany)

Friday, Sep 07

Session III: Updating the Y chromosome phylogeny
Chair person: Lutz Roewer

09h00 - 09h30 **Y-chromosomal insights from large-scale resequencing**
Chris Tyler Smith (Cambridge, United Kingdom)

09h30 - 09h45 **A calibrated human Y-chromosomal phylogeny based on resequencing**
Wei Wei, Ayub Q, Chen Y, McCarthy S, Hou Y, Carbone I, Xue Y, Tyler-Smith C (Cambridge, United Kingdom)

09h45 - 10h00 **Insight into human Y chromosome variation from low-coverage whole-genome resequencing data**
Yali Xue, Chen Y, McCarthy S, Ayub Q, Jostins L, Durbin R, Tyler-Smith C (Cambridge, United Kingdom)

10h00 - 10h15 **Determining the phylogenetic position of Y-chromosomes based on whole genome SNP calling**
Anneleen van Geystelen, Decorte R, Larmuseau MHD (Leuven, Belgium)

10h15 - 10h30 **Increasing phylogenetic resolution still informative for Y-chromosomal studies on West-European populations**
Marten HD Larmuseau, Vanderheyden N, Van Oven M, Kayser M, Decorte R (Leuven, Belgium)

10h30 - 10h45 **Multiple recurrent mutations at four Y-chromosomal single nucleotide polymorphism sites in a 37 bp sequence tract on the ARSDP1 pseudogene**
Harald Niederstätter, Berger B, Erhart D, Gassner C, Schennach H, Parson W (Innsbruck, Austria)

10h45 - 11h00 **Mapping the evolving Y-SNP phylogeny for building consistent Y-SNP databases**
Sascha Willuweit, Roewer L, Niederstätter H, Geppert M (Berlin, Germany)

11h00 - 11h15 **Discussion** (Mod. Sascha Willuweit)

11h15 - 11h45 **Coffee break**

Session IV: Applications of the Y chromosome phylogenetic approach
Chair person: Daniel Corach

11h45 - 12h00 **Continent-wide decoupling of Y-chromosomal genetic variation from language and geography in native South Americans**
Michael Nothnagel, Roewer L, Gusmão L, Gomes V, González M, Corach D, Sala A, Alechine E, Palha T, Santos N, Ribeiro-dos-Santos A, Geppert M, Willuweit S, Nagy M, Zweynert S, Baeta M, Núñez C, Martínez-Jarreta B, González-Andrade F, Fagundes de Carvalho E, Aparecida da Silva D, Jose Builes J, Turbon D, Lopez Parra AM, Arroyo-Pardo E, Toscanini U, Borjas L, Barletta C, Ewart E, Santos S, Krawczak M (Kiel, Germany)

12h00 - 12h15 **Identification of a novel Native American Y chromosome founding lineage in Northwest South America**
Lutz Roewer, Nothnagel M, Gusmão L, Gomes V, González M, Corach D, Sala A, Alechine E, Palha T, Santos N, Ribeiro-dos-Santos A, Geppert M, Willuweit S, Nagy M, Zweynert S, Baeta M, Núñez C, Martínez-Jarreta B, González-Andrade F, Fagundes de Carvalho E, Aparecida da Silva D, Jose Builes J, Turbon D, Lopez Parra AM, Arroyo-Pardo E, Toscanini U, Borjas L, Barletta C, Ewart E, Santos S, Krawczak M (Berlin, Germany)

12h15 - 12h30 **Y-haplogrouping among ethnic minority groups in South America**
Toshimichi Yamamoto, Sakuma M, Kawaguchi Y, Kano Y, Danjoh I, Nakamura Y (Nagoya, Japan)

12h30 - 12h45 **Evaluating social and ethical issues in Argentinean population by genetic marker analysis**
Daniel Corach, Alechine E, Caputo M (Buenos Aires, Argentina)

12h45 - 14h00 **Break**

14h00 - 14h15 **Tracking the Iceman's scent by high resolution mapping of Y haplogroup G in Tyrol (Austria)**
Burkhard Berger, Niederstätter H, Erhart D, Gassner C, Schennach H, Parson W (Innsbruck, Austria)

14h15 -14h30 **Beyond the migration: the Basque diaspora in the western USA**
Laura Valverde, Rosique M, García A, Köhnemann S, Cardoso S, Odriozola A, Pfeiffer H, M de Pancorbo M (Vitoria-Gasteiz, Spain)

14h30 - 14h45 **The history of Slavs in the light of Y chromosome and mtDNA variability**
Mielnik - Sikorska M, Daca P, Marcin Woźniak, Malyarchuk BA, Derenko MV, Skonieczna K, Grzybowski T (Bydgoszcz, Poland)

14h45 - 15h00 **Y-chromosomal STR analysis in Pashtun populations from Southern Afghanistan**
Niaz Muhammad Achakzai (Lahore, Pakistan)

15h00 - 15h15 **Y-chromosomal phylogeny of Porja and Savara Lineages in Andhra Pradesh, India**
Rao A. Isukapatla, Pulamaghatta NV, PBSV P, Adimoolam C (Mysore, India)

15h15 - 15h30 **Migration distance rather than migration rate explains genetic diversity in patrilocal groups**
Levy H, Conrado M, Montinaro F, Cristian Capelli
(Oxford, United Kingdom)

15h30 - 16h00 **Coffee break**

Session V: Next generation Y-STRs
Chair person: Manfred Kayser

16h00 - 16h30 **Rapidly mutating Y-chromosomal STRs**
Manfred Kayser, Ballantyne K, Ralf A on behalf of the RM Y-STR Study Group (Rotterdam, Netherlands)

16h30 - 16h45 **Development criteria for a next generation Y-STR multiplex for forensic applications**
Christina Bormann Chung, Mulero J, Nguyen V, Calandro L, Hennessy L (Foster City, USA)

16h45 - 17h00 **The PowerPlex® Y23 System: a single system for casework and database (direct amplification) Y-STR analysis**
Thompson JM, Ewing MM, Fulmer PM, Rabbach DR, Sprecher CJ, Douglas R. Storts (Madison, USA)

17h00 - 17h15 **Y-Chromosome specific nested PCR pre-amplification method for improved detection of male DNA**
Erin Hanson, Solivan M, Strauss S, Di Pasquale F, Engel H, Ballantyne J (Hilden, Germany); presented by Scherer M

17h15 - 18h15 **Panel discussion with companies and researchers** (Manfred Kayser, Chris Tyler-Smith; Life Technologies, Promega, Qiagen;
Chair person: Lutz Roewer)

Social Evening
Sponsoring Life Technologies

Saturday, Sep 08

Session VI: New technologies in haploid marker analysis
Chair person: Peter de Knijff

09h00 - 09h30 **mt-GoNL: deep sequencing of 750+ complete Dutch mtDNA genomes**
Peter de Knijff, Vermaat M, Li M, van Oven M, de Dunnen JT, Stoneking M, Kayser M, Laros J (Leiden, Netherlands)

09h30 - 09h45 **Applications of Personal Genome Machine (PGM™) in SNP-based human identification**
Sharon C Wootton, Langit R, Lagacé R, Hennessy L (Foster City, USA)

09h45 - 10h00 **Next generation mtGenome sequencing for forensic purposes using the Ion Torrent PGM**
Strobl C, Huber G, Lagace R, Langit R, Wootton S, Hennessy L, Walther Parson (Innsbruck, Austria)

10h00 - 10h15 **The impact of PCR and DNA sequencing artifacts in 454 LifeScience data on the interpretation of mtDNA heteroplasmy**
Mitchell M Holland (State College, USA)

10h15 - 10h30 **Weighing the differences: the application of mass-spectrometry for mtDNA control region analysis**
Mayra Mayr-Eduardoff, Huber G, Zimmermann B, Bayer B, Schmid D, Anslinger K, Göbel T, Niederstätter N, Schneider PM, Röck AW, Parson W (Innsbruck, Austria)

10h30 - 11h00 **Coffee break**

Session VII: Biostatistics of lineage markers
Chair person: Michael Krawczak

11h00 - 11h15 **Interpretation of lineage markers**
Bruce S Weir, Aalbers S (Seattle, USA)

11h15 - 11h35 **Evidentiary strength of a rare haplotype match**
Charles H Brenner (Oakland, USA)

11h35 - 11h55 **Estimating trace-suspect match probabilities for singleton Y-STRs haplotypes using coalescence theory**
Mikkel M Andersen, Caliebe A, Jochens A, Willuweit S, Krawczak M (Aalborg, Denmark)

11h55 - 12h30 **Discussion**

12h30 - 12h45 **Y-STR mutations: what paternity cases can tell us about the relationship between allele length and mutation rate**
Arne Jochens, Caliebe A, Roesler U, Krawczak M (Kiel, Germany)

12h45 - 13h00	**Testing Y-chromosome STR mutation rates using deep-rooting pedigrees** Johannes C Erasmus (Pretoria, South Africa)
13h00 - 13h15	**Analysis of mutation rates in purported brother pairs with 17 Y-STR loci** SM Edson, Maynard L Kerry (Dover AFB, USA)
13h15 - 13h30	**Searching for dependencies between mitochondrial and Y-chromosome DNA markers** Paulina Wolanska-Nowak (Krakow, Poland)
13h30 - 13h45	**Mine, yours, ours? sharing data on human genetic variation** Giovanni Destro Bisol, Anagnostou P, Capocasa M, Congiu A, Milia N, Montinaro F, Sanna E (Rome, Italy)
14h00	**Closing of Meeting**

Posters

Poster Sessions are held during all coffee breaks

Thursday: 16h30 - 17h00
Friday: 11h15 - 11h45 and 15h30 -16h00
Saturday: 10h30 - 11h00

mtDNA Phylogeny

P 1 **mtDNA haplogroup F1a'c is a genetic risk factor for nasopharyngeal carcinoma in Chaoshanese**
Du J, Deng J, Yao Y, Lin K, Chen S, Hu S*

P 2 **Mitochondrial DNA polymorphism and matrilineal genetic composition of Chaoshan population in China**
Hu S*, Deng J, Feng G, Du J, Chen S

P 3 **Insights into South-American colonization through mtDNA analysis in native Colombian populations**
Xavier C, Builles J, Gomes V, Ospino JM, Amorim A, Gusmao L, Goios A*

P 4 **Comparative mitochondrial DNA analyses in Myanmar and the distinct genetic position of the Karen people within this multi-ethnic population**
Summerer M, Horst J, Erhart G, Horst D, Horst B, Sanguansermsri T, Manhart A, Kronenberg F, Kloss-Brandstätter A*

P 5 **Multiplex mutagenically separated PCR assays for simple and rapid screening of East Asian mtDNA haplogroups on forensic samples**
Kim E, Lee H, Yoon J, Yang W, Shin K*

P 6 **Mitochondrial DNA data of five Philippine Negrito populations**
Tabbada KA, Salvador JM, Delfin FC, De Ungria MA*

P 7 **An approach to Equatorial Guinea demographic genetic history through maternal lineage analysis**
Valente C*, Alvarez L, López-Parra AM, Parson W, Amorim A, Prata M, Arroyo-Pardo E, Gusmão L

P 8 **Mitochondrial DNA data of Cabo Verde immigrant population living in Lisboa**
Afonso Costa H*, Morais P, Amorim A, Vieira Silva C, Matos S, Marques Santos R, Espinheira R, Costa Santos J

P 9 **Dissection of mitochondrial DNA haplogroup L3b and its forensic applications**
Pasino S*, Del Pero M, Santovito A, Robino C

P 10 **Refined characterization of Portuguese mtDNA variability for forensic purposes**
Rocha A, Goios A, Amorim A, Gusmao L, Alves C*

P 11 **MtDNA haplogroup distribution in Finland**
Putkonen M*, Neuvonen A, Hedman M, Palo JU

P 12 **Optimization and validation of a mitochondrial DNA assay in a German population sample and application to highly degraded DNA**
Zander J, Rothe J, Roewer L, Nagy M*

P 13 **MtDNA analysis of Mocoví population, southern most Guaycurú speakers in South America**
Sala A*, Martí MC, Bobillo MC, Corach D

P 14 **A very high level of Native American ancestry in an Amazon, Brazil, urban population inferred by mitochondrial DNA and indels markers analysis**
Hermida RM, Manta FS, Silva DA, Carvalho-Costa FA, Monteiro M, Moraes MO, Carvalho EF*

P 15 **Mitochondrial control region sequences of the Czech Republic population and a comparison to other populations**
Vanek D*, Silerova M, Urbanova V, Saskova L, Dubska J

P 16 **Insertion/delection polymorphisms in South Portugal Caucasian population: a preliminary study**
Vieira Silva C*, Matos S, Amorim A, Afonso Costa H, Morais P, Marques Santos R, Espinheira R, Costa Santos J

Y-chromosomal Phylogeny

P 17 **Internal validation of the AmpFlSTR Yfiler amplification kit and calculation of the genetic diversity of 17 Y-short tandem repeats in the Norwegian population**
Hellerud BB*, Lønning L, Heitmann I, Hansen EN

P 18 **Y chromosome diversity in the South and East Kazakhstan**
Tarlykov P*, Zholdybayeva E, Ramanculov E

P 19 **Genetic structure of the Y chromosome does not suggest intraregional sex-biased dispersals in the population of Asturias (Northern Spain)**
Cano-Garcia C, Pardinas A, Garcia-Vazquez E, Roca A, Lopez B*

P 20 **Y-STR haplotypes and the genetic structure of Pathan populations in FATA and NWFP of Pakistan**
Lee H, Sim J, Choi A, Rakha A, Yang W, Shin K*

P 21 **Development of six-SNPs assay for forensic analysis in European population**
Ferri G*, Ferrari F, Corradini B, Santunione A, Alù M

P 22 **Genetic journey of the N1c haplogroup**
Pamjav H*, Nemeth E, Feher T, Volgyi A

P 23 **Phylogeographic analysis of human Y chromosome diversity in eastern Africa**
Cruciani F*, Ippoliti M, Massaia A, D'Atanasio E, Moral P, Coppa A, Trombetta B, Sellitto D, Pascone R, Scozzari R

P 24 **Y-chromosome variation in geographically and linguistically isolated populations from oriental Alps**
Coia V*, Capocasa M, Scarnicci F, Boschi I, Anagnostou P, Battaggia C, Crivellaro F, Ferri G, Brisighelli F, Busby G, Capelli C, Destro-Bisol G

P 25 **Haplotype analysis of 23 polymorphic Y-STR markers in Northwest of Argentina**
López-Parra AM*, Mesa M, Brabo Ferreira Palha Td, Gusmão L, Lomaglio DB, Baeza C, García M, Marrodán M, Pacheco JL, Bejarano IF, Dip NB, Baillet G, Arroyo-Pardo E, Dipierri JE, dos Santos SE, Ribeiro dos Santos K

P 26 **Y chromosome DNA variation monitored in Slovakian populations by SNP and STR analysis**
Carnogurska J*

P 27 **Y chromosome diversity in Piedmont**
Caratti S*, Di Gaetano C, Matullo G, Gino S, Inturri S, Robino C

P 28 **Genetic analysis of 17 Y-STR loci in Pashtun population from Swat Valley, Pakistan**
Achakzai NM*

P 29 **Novel Y chromosome polymorphisms in Native American haplogroup Q1a3a1**
Alechine E, Corach D*

P 30 **Population data for 8 Y-chromosomal STRs (not included in Y-filer™ kit) in a population sample of Czech Republic**
Vanek D*, Saskova L, Silerova M, Dubska J

P 31 **Y-STR haplotype diversity of a native population of Cabo Verde living in Lisboa**
Marques Santos R*, Amorim A, Afonso Costa H, Vieira Silva C, Morais P, Matos S, Espinheira R, Costa Santos J

P 32 **Y-chromosomal STRs mutation analysis by the PowerPlex® Y23 system**
Ceccardi S, Riccardi LN, Fersini F, Lanzellotto R, Falconi M, Bini C*, Pelotti S

Comparative mtDNA/Y-chromosomal Phylogeny

P 33 **Determination of the Y chromosome DNA haplotype and mtDNA haplotype from 50 males from 17 countries**
Takasaka T*

P 34 **Forensic efficiency of combined Y/mt profiles in seven Iranian groups**
Bertoncini S, Farjadian S, Taglioli L, Ghaderi A, Romeo G, Luiselli D, Tofanelli S*

P 35 **Ancestry evaluation of Rio de Janeiro population by screening the Y chromosome and mitochondrial DNA**
Oliveira AM, Hermida RM, Silva DA, Gusmao L, Carvalho EF*

P 36 **Haploid markers in DNA identification process in Croatia**
Furač I*, Karija Vlahović M, Mašić M, Kubat M, Strinović D

Ancient DNA

P 37 **Forensic science applied on prehistoric remains - a nine fold burial of the 4th millennium BC raised questions about kinship, locality, and circumstances of death**
Friederich S*, Schlenker B, Stecher M, Bauer C, Niederstätter H, Parson W, Karimnia S, Meller H, Alt K

P 38 **Establishment of mitochondrial SNPs for the investigation of skeletal material buried in the ground**
Thiele K*, Pflugbeil A, Kohl M, Bruchhaus H, Dressler J

P 39 **Huns in Bavaria? Genetic analyses of an artificially deformed skull from an early medieval cemetery in Burgweinting (Regensburg, Germany)**
Schleuder R*, Wilde S, Burger J, Grupe G, Forster P, Harbeck M

P 40 **Y-chromosomal analysis of skeletal remains of a Swiss national hero from the 17th century**
Haas C*, Shved N, Rühli F, Papageorgopoulou C, Krawczak M, Willuweit S, Purps J, Roewer L

P 41 **Assessment of the origins of ancient Croatian remains through mitochondrial DNA interpretation**
Phillips LA, Fox A, Primorac D*, Holland M

P 42 **Genetic relationship between modern populations and the Neolithic Tyrolean Iceman**
Coia V*, Cipollini G, Maixner F, Brisighelli F, Capelli C, Battaggia C, Destro Bisol G, Zink A

P 43 **Molecular genetic analyses of skeleton excavated from Auersperg Chapel archaeological site in Slovenia**
Pajnič IZ*, Pogorelc BG, Balažic J, Horvat M

P 44 **DNA analysis of lineage markers (mtDNA and Y-chromosomal STRs) on ancient or aged bone samples**
Vanek D*, Saskova L, Urbanova V, Dubska J, Silerova M, Beran M

Short Tandem Repeat polymorphisms

P 45 **Forensic genetic analysis for the AmpFlSTR Yfiler system in the Korean population**
Kim S, Kim K, Han M, Kim W*

P 46 **Genetic polymorphisms of 19 STR Loci in the Chaoshan Han population**
Shang J, Hu S*, Chen S

P 47 **Central Croatian population data of eight X-linked markers in four linkage groups**
Gršković B*, Mršić G, Zidkova A, Vrdoljak A, Stenzl V, Popović M, Primorac D

Non human mtDNA

P 48 **Canine mitochondrial genome sequencing to improve the genetic profiling of dog hair**
Verscheure S*, Desmyter S, Backeljau T

P 49 **Is the Indian Wild Boar an evolutionary significant unit? Molecular insight into phylogeny of the Wild Boars and Domestic Pig**
Gupta S*, Hussain S, Singh L

New Technologies

P 50 **Investigator Plus – fast, sensitive and robust amplification of common standard set loci**
Prochnow A*, Scherer M, Steeger B, Pakulla S, Breitbach M, Cornelius S, Fischer C, Bochmann L, Schnibbe T, Engel H

P 51 **How to improve STR analysis using a novel quantification technology: more than DNA quantification**
Di Pasquale F, Cornelius S, König M, Bochmann L, Prochnow A*, Schnibbe T, Engel H

P 52 **Improving DNA sensitivity and strengthen reliability with low-level DNA testing using trace amount of metal**
Honda K*, Nishi T, Iwabuchi Y

P 53 **Sample lysis and DNA separation in single tube assemblies for accurate forensic profiling**
Schnerr H*

Statistics

P 54 **Estimating forensic match probabilities for Y-STRs using a new, discrete Laplace distribution**
Andersen MM*, Eriksen PS, Morling N

P 55 **Evaluation of the use of software as a tool in checking mitochondrial DNA HVI and HVII sequences analysis**
Funabashi K, Godoy C, Sousa M, Iwamura E*

P 56 **The effect of sample size on the estimates of mtDNA genetic diversity parameters in isolated European populations**
Anagnostou P*, Capocasa M, Montinaro F, Coia V, Destro-Bisol G

P 57 **Genes, mountains and culture: evidence for the impact of ethnicity on the structure of human populations**
Capocasa M*, Battaggia C, Anagnostou P, Montinaro F, Arena A, Boschi I, Brisighelli F, Capelli C, Coia V, Rufo F, Crivellaro F, Destro Bisol G

P 58 **Critique of the haplotype surveying method**
Brenner CH*

P 59 **Evaluation of haplogroup predicting softwares**
Caputo M, Bobillo MC, Alechine E, Sala A, Corach D*

Oral Presentations

Haplogrouping and phylogenetic analysis of mtDNA profiles

Bandelt H-J[1,*]

[1]Department of Mathematics, University of Hamburg, Hamburg, Germany

Phylogenetic principles are fundamental to genetic research and forensic case studies of mtDNA. Web resources and analytical tools for assessing phylogenetic relationships exist in abundance, which may seduce the user to regard this enterprise as a merely automatic procedure. In particular, haplogrouping of mtDNA in forensics has often been treated as a black box into which control-region data are fed and correct haplogroup assignments are returned. In reality, haplogrouping is part of a basal phylogenetic analysis with deficient information (mtDNA control region). Since most available programs suffer from several shortcomings, the user cannot rely on such tools without performing additional database searches and own phylogenetic analyses. The fine-classification of region-specific lineages not yet covered by PhyloTree can constitute a further challenge. Then supplementary sequencing of selected entire mtDNA genomes by high-throughput sequencing is a valuable strategy. However, the forensic field should avoid pitfalls that have occurred in molecular anthropology with most previously published data, which were automatically generated on Illumina platforms but without the necessary manual a posteriori analysis. Quasi-median networks, representing the duals of data tables, play a useful role in exploratory data analysis of mtDNA sequences. Since (full) quasi-median networks of total mtDNA data sets are often far too lage for visualization, one usually has to resort to either mutation-filtered data for focussing on potential sequencing artifacts or to sample-skimmed data for focussing on problematic parts of estimated mtDNA trees. Another option will be thinning of a quasi-median network by systematically removing highly ambiguous portions of the network, thereby modifying an earlier concept of "pruned median network".

A "Copernican" reassessment of the human mitochondrial DNA tree from its root

Behar DM[1,2,*], van Oven M[3], Rosset S[4], Metspalu M[1], Loogväli E[1], Silva NM[5], Kivisild T[1,6], Torroni A[7], Villems R[1]

[1]Estonian Biocentre and Department of Evolutionary Biology, University of Tartu, Tartu, Estonia
[2]Molecular Medicine Laboratory, Rambam Health Care Campus, Haifa, Israel
[3]Department of Forensic Molecular Biology, Erasmus MC University Medical Center Rotterdam, Rotterdam, Netherlands
[4]Department of Statistics and Operations Research, School of Mathematical Sciences, Tel Aviv University, Tel Aviv, Israel
[5]Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Porto, Portugal
[6]Leverhulme Centre for Human Evolutionary Studies, Department of Biological Anthropology, University of Cambridge, Cambridge, United Kingdom
[7]Dipartimento di Genetica e Microbiologia, Università di Pavia, Pavia, Italy

The principle of nested hierarchy is fundamental in our phylogenetic interpretation of the Tree of Life. At the gene level, this means that all polymorphisms known in any given genetic loci contribute to its ever-evolving phylogeny as reflected by their sequential accumulation in a tangled ancestry-descendant relations since the time of the most recent common ancestor (MRCA). Accordingly, the reconstructed ancestral sequence - the root - of any genetic locus should also serve as the reference point for its respective phylogeny. Hence, the use of an arbitrary chosen reference sequence within a rooted phylogeny leads to an inevitable conceptual clash between its nomenclature scheme and very essence. The Human mitochondrial DNA (mtDNA) phylogeny is a molecular evolution prototype of a haploid nonrecombining genetic locus, and knowledge about its variation has been extensively used in population genetic, medical, genealogical and forensic studies. Yet, all mutations comprising it were traditionally identified relative to the rCRS, a recently derived reference sequence, creating an obvious inconsistency when compared to their actual accumulation throughout the course of evolution. Recently, we have proposed switching to a Reconstructed Sapiens Reference Sequence (RSRS) as the only phylogenetically valid reference. The established Human mtDNA phylogeny root and the available H. Neanderthalensis complete mtDNA sequences allowed us to set the RSRS with confidence, avoiding frequent misunderstandings and direct errors resulting from the highly arbitrary nature of the used hitherto reference, thus, establishing an intellectual cohesiveness with the current consensus of shared common ancestry of all contemporary human mitochondrial genomes. To facilitate data transition, the website www.mtdnacommunity.org was established and is committed to the support of the "Copernican" reassessment of the human mtDNA phylogeny and to the establishment of computational tools meant to facilitate phylogenetic analysis and comparison of complete mtDNA sequences. The website allow an easy transition from an rCRS to an RSRS based nomenclature, automatically labels haplogroups, performs a phylogeny based quality check, identifies private substitutions, and compares new sequences with previously stored mitogenomes to suggest the labelling of additional haplogroups.

PhyloTree - growing and refining the human mitochondrial DNA tree

van Oven M[1,*]

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PhyloTree (http://www.phylotree.org) provides an up-to-date phylogeny of human mitochondrial DNA [1], and is now widely used by the forensic, population-genetic and medical research communities. Since its inception in August 2008, PhyloTree has been updated 13 times and the number of labeled nodes (haplogroups) has increased from 1,194 in Build 1 to 3,372 in Build 14, representing an almost threefold resolution gain. The tree is inferred from all available complete mtDNA sequences from the literature (numbering 15,451 as of May 1, 2012) following the principle of maximum parsimony. Haplogroup nomenclature follows the literature as much as possible and is extended where needed; in a number of cases, however, haplogroup nomenclature was revised such as in haplogroup M9 [2].One of the main applications of PhyloTree is haplogroup assignment and several tools for automated haplogrouping have adopted PhyloTree as the underlying classification tree (e.g. HaploGrep, mthap, HmtDB). Furthermore, the tree facilitates the selection of suitable SNPs for multiplex genotyping tools [e.g. 3].Recently, the inferred mtDNA root sequence, the RSRS, was proposed as an evolutionary more valid reference sequence than the rCRS [4]. Concomitantly, in Build 14, the way of denoting mutations was adjusted to include the ancestral and derived alleles relative to the root. As this caused some inconvenience for those used to the 'original' mutation notation, an rCRS-oriented version of PhyloTree is still available in parallel.

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The EMPOP concept for reporting mtDNA haplogroups in forensic genetics

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Human mitochondrial DNA (mtDNA) has proven to be a valuable resource in forensics and population genetics. Increased availability of mtDNA data enables a deeper understanding of the mitochondrial phylogeny. It has been shown that the assignment of haplogroups to individual samples may be helpful to both understand the phylogeny and aid the detection of idiosyncrasies in mtDNA data. A curated, standardized and continuously updated phylogenetic tree is available to the community via Phylotree.org, however, current software solutions for assigning haplogroup information to mtDNA sequences do not specifically target forensic needs. Furthermore, haplogrouping that is solely based on a phylogenetic tree suffers from accurateness or the absence of highly recurrent mutations that are often neglected in the phylogenetic tree. We here present an approach tailored to particular needs of forensic genetics. Combining the alignment-independent search engine SAM of EMPOP with high-quality haplogrouped mtDNA sequences of the database allows for reliable haplogroup assignment of forensic samples via EMPOP. Using EMPOP as underlying data pool in addition to a phylogenetic tree allows for incorporating the maximum amount of information. Software-based determination of forensically relevant haplogroups accounts for the stability needed in forensic settings. Besides an additional quality control instrument this facilitates a more accurate assessment of the strength of evidence by providing haplogroup distributions as additional phylogeographic information.

Improved visibility of character conflicts in quasi-median networks

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Quasi-median network construction of reduced and filtered haplotypes provides a valuable tool for graphical representation of mitochondrial DNA data (Bandelt and Dür 2007, Parson and Dür 2007, Schwarz and Dür 2011). Errors, artefacts and ambiguities present in the data may induce character conflicts that increase the complexity of the network, pinpointing first approaches for quality control of data sets (Turchi et al 2007; Prieto et al 2011). However, indecipherable reticulations due to samplesize or errors cannot be interpreted even by the trained eye.

For that reason we enhanced the editing tool of the network layout by implementing new features and redesigned the graphical user interface. The major improvement of the new network editor includes the possibility to highlight the induced sub graph with concurrent dimming of its complement using mouse-over. This enables visual emphasis on substructures within a complex network. Via mouse click on a network node a list of the included haplotypes is generated, which allows direct inspection of the phylogenetic background. This new feature enables immediate and simple identification and inspection of the respective haplotypes and mutations.

The new editor is demonstrated by means of population data from West Eurasia and from East-Asia that were submitted to EMPOP (www.empop.org) for quality control. The new network editor is available on EMPOP for registered users.

Evaluating mtDNA profiles: Even the smallest difference has a size

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The most applied way of mtDNA profile interpretation in forensics is straight forward, and hence easy to apply in case work. When comparing profiles, two differences do exclude and equal profiles perform a match. The intermediate situation, with only one difference between the profiles, is considered as inconclusive. Nevertheless its simplicity, this approache neglects completely the mutation-specific weight and can be confusing in court room. Confronted with a single difference between mtDNA profiles in case work, extending the HV1-HV2 sequence to the complete control region doesn't always result in an exclusion. Further profiling of informative positions in the coding region is for most laboratories not an option due to analytical and/or legal limitations. Instead of reporting an mtDNA evaluation as inconclusive the DNA commission of the ISFG and the EDNAP group proposed, already more than ten years ago, to consider the size of the smallest difference between the profiles involved (Carracedo et al. 2000, Tully et al. 2001). On the basis of real case work examples we will demonstrate the influence of this consideration on the mtDNA profile interpretation. Therefore data were retrieved from the EMPOP database in its current state, and site-specific mutation rates were used for the calculation of the likelihood ratio.

Linguistic isolates in Portugal: insights from the mitochondrial DNA pattern

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The Miranda do Douro municipality, located in the northeastern region of the Portuguese territory, has notable characteristics not only from a geographic or naturalistic point of view, but also from a cultural perspective. One of its remarkable cultural traits is the coexistence of two different languages: Portuguese and Mirandês, a Leonese dialect. The current persistence of the Leonese dialect in this population falls on the singularity of the region: relative isolation implying difficulties to communicate with other Portuguese regions and the establishment of social and commercial relationships with adjacent Spanish territories. The objective of this study was to characterize this population through the analysis of its maternal lineages composition to address its phylogenetic origin in relation to other previously analysed populations in the Iberian Peninsula. In order to accomplish this purpose, mitochondrial DNA from 121 individuals was analyzed. A 3,348 bp mtDNA fragment was amplified and sequenced in two overlapping fragments using mitochondrial-specific primers to obtain a clear range electropherogram of the entire control region (16,024-576 bp, according to the rCRS). Haplogroup classification was performed following current nomenclature. The obtained results showed a haplogroup composition typically from a Western European population, with more than half of individuals (61.2%) classified into the macrohaplogroup R0. We also reported several lineages ascribed to an African (L2a and L1b), Indian (M5a1) and Jewish (N1b) origin, which together account for the 10.6% of the total variability detected. Both genetic and nucleotide diversities presented low values (0.9328 ± 0.0172 and 0.01110 ± 0.00619, respectively) when compared to its microgeographical framework. It should be pointed out that 48% of our samples were private lineages, which let us to hypothesize that the diversification of some of them could have occurred in the Mirandese region. The observed pattern of mtDNA variability matches with an isolation-related hypothesis for the survival of the Mirandese speaker population.

Genetic microdifferentiation patterns in the mitochondrial DNA pool of Asturias (Northern Spain)

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The mitochondrial DNA composition of the autonomous community of Asturias, in Northern Spain, has not been extensively studied. Its convoluted history, characterised by prolonged periods of isolation, makes it an interesting target for such a research. For this purpose, full mtDNA control region sequences were obtained from 361 volunteers with at least two generations of Asturian maternal ancestry. Places of birth of the maternal grandmothers of the volunteers were recorded, and sequences were grouped according to historical regions derived from ancient feudal lordships. A SAMOVA analysis was used to define maximally-differenced population groups based on genetic and geographic data. Results showed a significant between-group variation of 1%, without significant within-group or within-population differentiation. Such a clear genetic structuring is striking, and was further investigated by means of BARRIER and MIGRATE analyses. These showed the existence of two possible barriers inside the community and several instances of asymmetric gene flow between the SAMOVA-defined groups. All this data, combined with historical information, suggested the existence of two different processes as the cause of the structuring: Recent external migrations in the coastal north, probably related to increased commercial activity during the Middle Ages; and ancient isolation in the mountainous south, probably related to the rough terrain and cultural aspects of its population.

A detailed phylogeny of rare mitochondrial DNA haplogroups from negrito populations of Southeast Asia

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The geographic distribution and deep branching structure of the human phylogeny retains signals for a dispersal of humans from Africa to Australia during the late Pleistocene. The presumed route of migration was through what is now peninsular and island Southeast Asia, but at times of reduced sea levels would have been an almost continuous land mass separated by short sea crossings. At the time of European contact, there existed many foraging populations throughout the region, which share a distinct phenotype of small stature, dark complexion, and tight curly hair. These features led to them being known collectively as negritos, from the Spanish diminutive for black. Implicit in this name is the hypothesis that they share a common evolutionary history, representing an early substrate of humans in Southeast Asia, who have survived in isolated or marginal environments. Phylogenetic studies of mitochondrial DNA from negrito populations point to different axes of influence between Southeast Asia and adjoining regions. In the Westthe Andaman Islanders are argued to have been the subject of long-term isolation after an early settlement from South Asia, whilst the genetic diversity of those in the Philippines in the East contains haplogroups that appear to be be autochtonous to the region as a whole.

By sequencing whole mitochondrial genomes, we obtained detailed phylogenies of rare mitochondrial haplogroups in the Andaman and Philippine negrito populations and placed them in a regional phylogeographic context. The branching structure of haplogroups from the Andamans are consistent with an origin within Southeast Asia and support inferences made from nuclear DNA for a subsequent back-migration to adjacent parts of South Asia. The haplogroups specific to Philippine negritos, in contrast, indicate maternal connections to a population ancestral to present-day Melanesians and Australians. By adding 25 novel sequences to a data set of over 400 Eurasian whole mitochondrial genomes, we used a Bayesian statistical approach to estimate the dates of key nodes in the phylogeny using multiple internal calibrations. The results from this analysis provide evidence for internal diversification of the haplogroups within Southeast Asia ~40 ka, settlement of the Andamans at approximately half this time depth, and an upper limit for a possible back-migration to India of ~13 ka. These findings are discussed with reference to previous studies arguing for a settlement of the Andamans up to 65 ka, and admixture between ancestors of Philippine negritos and another species of hominin.

Regional sampling connects population genetics with forensics – an example from South America

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For most parts of the world, the “big picture” of human dispersal conveyed by population surveys of mitochondrial DNA (mtDNA) variation is well understood. The smaller the scale, the more details remain to be clarified within this framework due to the lack of extensive sampling and high quality sequencing of utmost large segments of the mitochondrial genome. With the ever growing number of worldwide sample contributors and the advanced technologies available, a regional concept has become realistic. By using as an example the recent discovery of two Native American mtDNA haplogroups (D1g and D1j) whose spatial distributions are restricted to the Southern Cone of South America, we illustrate the possibilities of regional sampling: the detailed investigation of a restricted area or population group and the small-scale geographic and ethnic dispersal of lineages may open a window to the past by revealing details about timing and routes of migration; and help to understand a native group’s history. In addition to delivering a more complete coverage of natural variation in databases, the regional concept of sampling is of highest importance in forensic applications of mtDNA (and other markers), as rarer lineages will not only tend to be missing in (small) countrywide samples, but local frequencies may also differ significantly from those in a general dataset. Therefore, major regional sampling and sequencing efforts are still mandatory for uncovering all (even the most basal) variation in mtDNA haplogroups, for reconstructing the details of migration processes, and for yielding correct frequency estimates, by targeting, when possible, both the general mixed population and autochthonous groups.

Genetic discovery of early medieval human skeletal remains based on gender and mtDNA data

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In 2001/02 the municipal Archaeologie in Hall accomplished by order of the Institute of Archaeologies (University of Innsbruck) successful excavations of an early Middle Age cemetery (dated between the 6th and 12th century CE) in Volders (Tyrol), which was a major settlement area in the Inn Valley since pre-historic times. On the basis of radiocarbon dating and grave situations, the majority of burials are assumed to range from the late 6th to the early 7th century. For this region and time period only scarce historical information is available. This cemetery represents one of the largest ancient series of human remains in Tyrol and the Alpine region in general. Of scientific interest is the burial situation in a tight area and the orientation of the skeletons with a prevalent restriction to East-West and only few North-South directions. Because of their excellent preservation state the historical remains lend themselves perfectly to the application of biomolecular methods to gain increased insight for a better interpretation of the findings. We present first comparative results of gender estimation by morphological and genetic methods and provide an overview of mtDNA typing results using Sanger Sequencing and mass spectrometry.

Forensic science applied on prehistoric remains - a nine fold burial of the 4th millennium BC raised questions about kinship, locality, and circumstances of death

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A monumental earth construction of the Salzmünde culture (3400-3025 BC) with 165 burials was discovered during excavations at the eponymous site Salzmünde in Saxony-Anhalt, Germany. Especially a multiple burial with potsherd filling that contains four adult women and five subadults raised questions about kinship, locality and cause of death. This exceptional ninefold burial is the matter of a transdisciplinary and integrative project combining archaeological, anthropological, stable isotope and palaeogenetic analyses. Our aim is to shed light on the relationships of the individuals of the ninefold burial and the circumstances that lead to the death of these people. In order to evaluate biological kinship DNA was extracted from bone and tooth samples of seven individuals. Mitochondrial haplotypes and haplogroups were identified by sequencing of the hypervariable segments I and II of the control region and by analyzing 22 diagnostic coding region single nucleotide polymorphisms. We were able to obtain reproducible endogenous DNA from all individuals investigated. Among these seven individuals we found three different mitochondrial lineages ascertained to distinct haplogroups suggesting maternal kinship among the individuals in the ninefold burial. Combining the results of every discipline of the ongoing project, it is currently not possible to define the circumstances of death. However, several burn marks on the bones of the individuals as well as other signs of violence seem not to be caused by a catastrophe and lend support for a violent raid or a ritual mortuary practice. Further analyses will show, whether the Salzmünde people have been victims of an act of war or ritual practices.

Different mutational spectra of complete mitochondrial genomes of normal and colorectal cancer cells

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Although mitochondrial DNA variability in cancer cells was widely studied by medical researchers, the reliable spectrum of mitochondrial DNA mutations in carcinogenesis is still unresolved. Therefore, variability of the 100 complete mitochondrial genome sequences of tumor and matched non-tumor tissues from 50 patients diagnosed with colorectal cancer were investigated. Comparison of the whole mitochondrial DNA sequences of tumor and non-tumor tissues from the same patient revealed somatic mutations in 82% of patients. Most of the somatic mutations in mitochondrial DNA of cancer cells hit evolutionary stable positions in human mtDNA phylogeny. Moreover, the most variable positions in control region reported in non-tumour cells were found to be the least prone to somatic substitutions in cancer cells. Slightly higher frequency of somatic mutations was found in the most conserved tRNA and rRNA genes, while the highest variation was found in protein coding gene region. In particular, the relatively most evolutionarily stable nd3 gene underwent somatic mutations with the highest frequency, and a relatively highly variable nd1 gene remained unchanged in the tumor cells. Somatic substitutions observed in tRNA and rRNA genes were predominantly located in stem regions. Moreover, majority of the somatic substitutions located in protein coding genes lead to amino acid changes in the polypeptide sequence, and more than half of them had a higher pathogenicity score from that observed for mutations associated with mitochondrial diseases. The neutrality test based on the analysis of protein-coding genes sequence variability showed relaxation of negative selection in mitochondrial DNA of cancer cells. Therefore, these results suggest that somatic mutations might have been introduced into mtDNA of cancer cells due to the their independence of oxidative phosphorylation.

MtDNA haplogroups and sudden infant death syndrome

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Between the years 1998 and 2001 cases of sudden infant death syndrome (SIDS) were sampled at the Institute of Legal Medicine in Münster. MtDNA haplogroups were investigated in 232 of these cases using frozen tissue of medulla oblongata or FFPE tissue of lung or heart. The results were compared to 148 saliva samples of healthy children with an age between one to five years. The results were as well compared to 842 samples representing the common mtDNA haplogroup distribution of the German population. This study will be discussed according to the question, whether mtDNA haplogroups are a predisposing factor for SIDS or not.

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Y-chromosomal insights from large-scale resequencing

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Next-generation sequencing technology now makes it possible to resequence whole genomes or targeted regions on a population scale, providing extensive sequence data from the Y chromosome. Coverage of the Y chromosome is lower than that of autosomes, and repeated sequences complicate mapping of reads to their correct location, but about 10 Mb of unique Y sequence is accessible to current technologies. We have explored the insights that can be obtained from two such datasets. Complete Genomics have released high-coverage sequences of 35 diverse males (http://www.completegenomics.com/), which we supplemented by sequencing an additional male belonging to haplogroup A. From these sequences, we identified about 6.6 thousand Y variants, which showed high validation rates. These variants were used to construct a maximum parsimony phylogenetic tree that recapitulated the known phylogeny and distinguished all individuals. Using a measured SNP mutation rate of $1x10^{-9}$ per bp per year, the ages of nodes of interest could be estimated. The TMRCA of the entire tree was ~115 KYA (thousand years ago), and of the lineages outside Africa ~60 KYA, both as expected. Additional insights included a rapid expansion of hg F ~40 KYA, and of R1b in Europe ~5-10 KYA. The archaeological counterpart of the former is unclear, but the latter is likely to represent a Neolithic expansion of this lineage. The second dataset consisted of low-coverage (~2x) sequence of 525 diverse males from the 1000 Genomes Project (http://www.1000genomes.org/). About 18.7 thousand Y-SNPs were called, >98% of which validated, but the callset missed ~17% of SNPs because of the low coverage. A maximum likelihood tree was constructed that again recapitulated and refined the known phylogeny and distinguished all individuals. The expansions noted above were also seen, although estimating times was more complex because of the missing variants. These explorations of large-scale Y resequencing illustrate the power and limitations of current technologies and also the need for the community to develop efficient ways to use such large datasets, including a nomenclature compatible with complete lineage resolution.

A calibrated human Y-chromosomal phylogeny based on resequencing

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We have analysed a dataset of 36 complete Y-chromosomal sequences, 35 released by Complete Genomics (http://www.completegenomics.com/) and an additional sequence from a haplogroup A3b individual, in order to explore how effectively complete sequence data from the Y chromosome can be used to construct and calibrate a phylogeny. We identified unique-sequence regions of the chromosome where we expected variant identification from next-generation sequence data to be reliable, and developed additional filtering steps for the data. Validation rates of the resulting filtered genotype calls were >99%. In total, we identified 5,865 SNPs, 741 indels and 56 MNPs. 4,861 of the variants are new and 262 of them are recurrent even in this small sample. We constructed parsimony-based phylogenetic trees using PHYLIP incorporating all or different subsets of the variants, and estimated times for the entire tree and different clades of interest using GENETREE or the rho measure. The tree structure was consistent with literature data. The GENETREE TMRCA for the complete set of chromosomes examined was 105-125 KYA; times for the out-of-Africa movement were 62-79 KYA, a Paleolithic expansion 37-48 KYA, and the expansion of R1b in Europe 7-10 KYA; rho times were broadly similar. Our study identifies vast numbers of new variants, and explores the methodological steps necessary to obtain reliable biological insights from current next-generation sequence data. It also poses challenges such as how to develop a nomenclature system that can accommodate such extensive sequence information, or how to identify the archaeological counterparts of the male expansions detected.

Insight into human Y chromosome variation from low-coverage whole-genome resequencing data

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Phase 1 of the 1000 Genomes Project has generated low-coverage whole-genome sequence data from 1,094 individuals from worldwide populations, including 528 males. SNP calls on the Y chromosome were made using SAMtools. In low coverage data, there are errors and uncertainty in the genotype calls. We developed a filtering strategy to reduce these, including restricting the analysis to 8.9 Mb of Y unique regions. We called a total of 18,692 Y-SNPs, 16,679 with the ancestral allele known. The false negative rate and false positive variant site identification rates were measured at 14% and 1.72% respectively by comparison with Complete Genomics calls on an overlapping subset of samples. The genotype accuracy was 97.4% compared with HapMap3 chip genotypes and 96.6% compared with Complete Genomics sequences. Using known literature variants, we assigned each sample to a haplogroup and these samples covered most of the major lineages except F, K, L, and M. A phylogenetic tree was constructed based on all the sites with known ancestral states using the RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analysis. The tree was consistent with the established structure. It confirmed Hg E (Bantu), O (China) and R1b (Europe) expansions associated with the Neolithic transitions in different parts of the world, and revealed that the expansion in Europe was the most extreme. One novel finding was a striking expansion of lineages F to R ~20 thousand years after the out-of-Africa movement, suggesting a previously unknown event of importance to male demography at this time.

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Determining the phylogenetic position of Y chromosomes based on whole genome SNP calling

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Due to the rapid progress of next generation sequencing (NGS) facilities, an explosion of human whole genome data will become available in the coming years. These data can be used to optimize the phylogenetic Y-chromosomal tree and to find new genetic lineages. Moreover, the exponential growth of known Y-chromosomal lineages will require an automatical determination of the phylogenetical position of a sequenced Y-chromosome based on whole genome SNP calling data and an up-to-date Y-chromosomal tree. Here, we will present a new software package, AMY-tree, which is able to determine the phylogenetical position of a Y-chromosome using a whole genome SNP profile. Moreover, it indicates ambiguities within the current phylogenetic Y-chromosomal tree based on several whole genome SNP profiles. This software program also points out new Y-SNPs which may be phylogenetically relevant in the future. AMY-tree works with SNPs which are called from whole genome sequencing data and it makes use of several complementary algorithms to determine the phylogenetical position of Y-chromosomes taking mistakes in the SNP calling and the (provisional) lack of good indel calling for full genome data into account. The AMY-tree software and all its capabilities were already tested in detail based on 36 male full genomes with different origins and the results will be presented during this talk.

Increasing phylogenetic resolution still informative for Y-chromosomal studies on West-European populations

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An increasing number of Y-chromosomal SNPs are becoming available besides the set of SNPs which were used to achieve the latest published phylogenetic tree of the human Y chromosome by the Y Chromosome Consortium (YCC) in 2008. Many Y-chromosomal lineages which are defined in this tree were mostly distributed in (Western) Europe due to the fact that most research projects are focusing on this area. Therefore the question arises if newly discovered polymorphisms on the Y-chromosome will still be interesting for Western Europeans on a population genetic level.

To answer this research question, the West-European region of Flanders (Belgium) was selected as study area since its Y-chromosomal variation and distribution are well known in detail. In this region, more than 1000 Y chromosomes which were genotyped at the highest resolution of the YCC-tree were coupled to the in-depth genealogical data of the autochthonous DNA donors. Based on these data the temporal changes of the population genetic pattern within Flanders are well studied for the last centuries, and the effects of several past gene flow events were identified.

Now, a set of recently published and newly developed Y-SNPs were optimized to characterize all Flemish Y-chromosomes belonging to haplogroups G, R1b and T. Based on this set, it was possible for the first time to observe a significant East-West gradient in the frequency of certain R1b Y-chromosomal lineages in addition to a previously announced North-South gradient. In this talk we will discuss therefore the informative value of recently discovered Y-SNPs for population genetic studies within Western Europe. The results suggest that an update of the Y-chromosomal tree based on new polymorphisms will give the opportunity to study population genetic patterns in more detail, even in an already well-studied region such as Western Europe.

Multiple recurrent mutations at four Y-chromosomal single nucleotide polymorphism sites in a 37 bp sequence tract on the ARSDP1 pseudogene

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The male-specific region of the human Y chromosome (MSY) escapes meiotic recombination and is passed down clonally from father to son. Mutation is the single driving force behind Y-chromosomal diversification. The slowly mutating binary Y-chromosomal single nucleotide polymorphisms (Y-SNPs) are considered the result of unique single base substitutions (unique event polymorphisms, UEPs) during human evolution. Sets of these binary MSY markers form stable paternal lineages that can be arranged to robust compound haplogroups. This enables the reconstruction of the Y chromosome's evolution by deducing a maximum parsimony haplogroup tree from the present-day MSY variation. The geographical distribution of MSY variation is non-random. Hence, Y-SNPs are of forensic interest, as they can be utilized e.g. for deducing the bio-geographical origin of biological material. This extra information can complement STR data in criminal investigations. For forensic applications, however, any targeted marker has to be unequivocally interpretable. Here we report findings from a population study comprising ~3,700 samples from Tyrolean men (Austria), indicating apparent homoplastic mutations for haplogroup R-M412, R-U152, and L-M20 Y chromosomes. The affected Y-SNPs P41, P37, L202, and L203 mapped to a 37 bp region on Yq11.21. Observing in multiple phylogenetic contexts up to four homoplastic mutations within such a short sequence tract hardly results from a series of parallel mutations. We rather propose heritable X-to-Y gene conversion as a more likely scenario. The four affected Y-SNPs should be considered gametologous sequence variants (GSVs) rather than UEPs. We also extracted information contained in the current ISOGG 2012 Y-SNP list and the human reference genome assembly to identify additional potential inter-chromatid gene conversion hotspots.

Mapping the evolving Y-SNP phylogeny for building consistent Y-SNP databases

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The increased awareness of the practical forensic use of Y-SNPs is accompanied by a lack of standardization of phylogenetic Y chromosome analyses. Since the last publication of the Y Chromosome Consortium (YCC, Karafet et al. 2008) plenty of new markers, branch names and corrections have been published in the academic literature. There is an electronic repository driven by International Society of Genetic Genealogy (ISOGG) that tries to combine results/information from the field of “genetic-ancestry testing” and of academic literature. Because forensic reporting crucially depends on the scientific acceptance, standardization and reproducibility of the applied methodology, this resource is not applicable as a reference here. Since 2008 the Y Chromosome Haplotype Reference Database (YHRD 3.0) includes Y chromosome reference samples typed for Y-SNPs and presents inferred haplogroups. To conform with the forensic standards it is necessary to guarantee a correct and up-to date nomenclature as well as scrutiny to eliminate incorrect typing results and to harmonize nomenclature. We take a case of an obvious homoplasy of the P37 mutation to propose necessary steps for the forensic validation of Y-SNPs a) Discussion and publication of guidelines on mandatory validation requirements for new Y-SNP markers published after 2008,b) Set-up of a central scientifically curated repository of validity states of Y-SNP markers, c) Proposal for a revised, unequivocal nomenclature which accounts for the current Y haplogroup phylogeny with synonymous markers and large numbers of branches (e.g. R-U152 instead of R1b1b2a1a2d).

Continent-wide decoupling of Y-chromosomal genetic variation from language and geography in native South Americans

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The way of initial habitation creates a primordial spatial pattern of human genetic variation that is subsequently modified by demographic processes. Under the assumption that most such changes follow trajectories set by climate as well as geographic and cultural conditions, some correlation between the genetic structure on the one hand, and the linguistic and geographical structure on the other, is to be expected in extant human populations even a long time after an initial colonization event and has been documented in numerous studies on European and Asian populations. In contrast, there is a notable absence of such descriptions for South America. Here, we examined Y-chromosomal genetic diversity and its relation to geographic location and linguistic classification on a continental scale in the so far largest study on South American natives, involving up to 17 microsatellite markers genotyped in a total sample of 1011 individuals representing 50 tribal populations from 81 settlements as well as single-nucleotide polymorphisms defining the founding native American phylogenetic lineages Q and C and its sub lineages. We observed a large decoupling of Y-chromosomal genetic diversity from geographical habitats and in parts from language groups on a continental scale, which is consistent with a rapid peopling of the continent and subsequent long periods of isolation of relatively small-sized tribal groups. Our results highlight the fact that a pronounced correlation between genetic and geographic/cultural structure can be expected only under very specific conditions, most of which are likely not to have been met by native South Americans.

Identification of a novel Native American Y chromosome founding lineage in Northwest South America

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For the first time, we could identify a novel Native American founding lineage C-M217 (C3*) within a restricted area of North-west South America. This finding is intriguing in view of the high prevalence of the same haplogroup in Central, East and North East Asia, and its concurrent absence from North America. Possible scenarios include (i) later migratory waves that quickly passed the existing populations in North America, and (ii) long-distance trade or contact with East Asia. Fifty years after Estrada, Meggers and Evans (1962) suggested trans-Pacific connections between the middle Jōmon culture of Kyushu (Japan) and the littoral Valdivia culture in Ecuador (6400-5300 YBP), based upon cultural similarity, it is indeed tempting to speculate that C-M217 (C3*) was introduced into South America from Eastern Asia by sea, either along the American West coast or across the Pacific (with some help by major currents). The striking differences observed between the Y-STR haplotypes of Ecuadorian and Asian C-M217 (C3*) carriers would be explicable in terms of a long divergence time after the arrival (although a more recent introduction cannot be excluded).

Y-haplogrouping among ethnic minority groups in South America

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RIKEN BRC preserves a valuable cell collection called “the Sonoda-Tajima Cell Collection” where cell samples collected from a variety of ethnic minority groups across the world, especially from South America. A part of the samples were immortalized, and the B-lymphoblastoid cell lines (B-LCLs) were established. Alternatively, it is important to investigate the genetic relationship and structure among the present ethnic groups in South America to obtain the information about the peopling of the Latin Americas. In the present study, we analyzed Y-haplogroups (Y-HGs) to investigate the origin, the genetic relationship, and the degree of admixture of male-linage.

We extracted DNA from the 204 B-LCLs originated from males among ethnic minority groups (23 groups in 8 countries, and missing) in South America. These DNA samples were analyzed for Y-HGs mainly by a method using a SNaPshot multiplex kit (Geppert, FSI genet.5:100, 2011), and additionally by an allele-specific PCR method by ourselves.

We observed 6 patterns of electrophrogram from all of the 204 DNA samples, 162, 18, 11, 10 and 3 of Y-HG-Q, -R, -E, -FtoJ and –K-toT, respectively by the major haplogrouping. One hundred and sixty-two samples of HG-Q were also divided into 2 sub-HG-Qs, 136 of Y-HG-Q1a3a* and 26 of Y-HG-Q1a3 by the Q-specific haplogrouping. Furthemore, by an allele-specific PCR method, 3, 1, 5 and 1 of Y-HG- G, -I, -J and –O, respectively were observed among 10 of Y-HG-FtoJ and 3 of Y-HG-K-toT. Consequently, all of 14 samples from Sanuma tribe in Venezuela were haplogrouped as Y-HG-Q1a3. I was indicated that this tribe is extremely isolated by 21 autosomal STRs. Alternatively, all of 7 samples from Saraguro tribe and about half of Canar tribe in Ecuador were not haplogrouped as Y-HG-Q. Therefore, it was suggested some ethnic minority groups in Ecuador are more mixed with European and/or African.

Evaluating social and ethical issues in Argentinean population by genetic marker analysis

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American continent countries are characterized by heterogeneous populations resulting from complex admixture events in which Native American, European and Occidental Sub-Saharan African contributors participated. The proportion of each continental contribution highly varies between the different American countries. The foreign, European conquerors and their African slaves -brought to the continent as working forces- determined an initial displacement of the aboriginals since the beginning of 15th century. After almost four centuries of interethnic interactions, during the second half of 19th and early 20th century a massive immigration, mostly from Europe, took place, impacting drastically on the already admixed local population. Aiming to analyze if any discrimination is operating in Argentina we investigated the frequency of Native American maternal and paternal lineages in two different scenarios: a-Paternity testing and b-Criminal identification. We analyzed 1024 samples donors by means of Y chromosome Q1a3a, R1b1b2 and I haplogroups and mtDNA hg A2, B2, C and D1. Concerning matrilineage alone, Native American-specific mt-hg proportions were significantly different between both groups (Paternity=72.9%, Forensic=79.8%, P=0.03). In addition, Y-hg analysis did also showed a significant difference in Native American hg Q1a3a proportion between forensic and paternity cases (Paternity=8.6%, Forensic=18% ;P=0.0009). As expected, when considering both maternal and paternal Native American lineages together, the difference between the groups under analysis was also statistically significant (Paternity=6.8%, Forensic=18%; p=0.0003).Our results clearly point that aboriginal population and their admixed descendants might have had less opportunities in their social and economical development leading the individuals with Native American ancestry to be marginalized and stigmatized being prone to criminal activities. Our results represent the first objective demonstration of nowadays discrimination in Argentina and might contribute to redefine this unacceptable social condition since all human beings inhabiting any piece of land deserve the same rights, the Human Rights.

Tracking the Iceman's scent by high resolution mapping of Y haplogroup G in Tyrol (Austria)

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The analysis of human Y chromosome variation is a well established tool for investigating human history on the basis of present-day genetic diversity. Most recently, progress in ancient DNA analysis makes direct comparisons with genetic data from archeological remains increasingly possible. For example, there is evidence from ancient genetic data for a high frequency of Y chromosomal haplogroup G (Hg) in prehistoric populations of Central Europe, whilst nowadays the density distribution of these Y chromosomes reaches its peak in the Caucasus. The most recent and most prominent example for these findings is the "Tyrolean Iceman". The 5,300 years old mummy was found in the Ötztal Alps near the border between Italy and Tyrol (Austria). Y chromosomal single nucleotide polymorphisms (Y-SNP) analysis assigned the Iceman to a sub-branch of G2a, which is defined by the SNP L91. This particular Hg is very rare in present-day populations of Europe (< 1%).

In this work we set out to test for elevated levels of G2a and in particular of G-L91 in present-day paternal lineages in the remote mountain regions near to the site where the Iceman was found. A population sample comprising 3,713 specimens from men living in Tyrol was genotyped for 19 Y-SNPs by single-nucleotide primer extension. This set included the G2a defining marker P15. Preliminary results indicated that app. 11% of the Y chromosomes belonged to G2a. The spatial distribution of this Hg featured unexpectedly high densities within or near the Ötztal Alps. L91 and additional SNPs such as L32, L487, and L645 are increasing the resolution within G2a and will refine this pattern.

Beyond the migration: the Basque Diaspora in the Western USA

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The population of the Basque Country has remained isolated from the arrival of new individuals until the second half of 20th century. However, it has been the source of several emigrant waves looking for new opportunities even in other continents. The Western USA received a large number of individuals, principally males, of Basque origin during the second half of the 19th century, who settled in Basque communities that nowadays still try to maintain their ethnic identity. The settling of a diaspora implies several phenomenons which can influence the genetic structure of the immigrant population. For one hand, the typical consequences of a migration, which are principally the founder effects, the interruption of the gene flow from the source population and the admixture with the receptor population. On the other hand, the consequences of the migration of this Basque population with strongly isolated ethnicity, which could facilitate the loss of panmixia. With the aim of studying the effects of this migration on the genetic structure of the paternal lineages in the Basque Diaspora in the Western USA, we have analysed 17 Y-STRs and 18 Y-SNPs in a sample set of individuals from this Diaspora and in a sample set of autochthonous individuals from the Basque source population. The results show that the Basque Diaspora in the Western USA largely conserves the Y-chromosome genetic legacy of the autochthonous European Basques, with no genetic substructure, no loss of diversity and a very small amount of new American Y-chromosomes incorporated to its gene pool.

The history of slavs in the light of Y chromosome and mtDNA variability

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To explore the origin of Eastern Europeans we investigated Y chromosome and mitochondrial H5 haplogroup diversity in population samples from Ukraine and other Eastern European countries. Y chromosome diversity was analyzed using a panel of 11 SNP polymorphisms (including M458 – so called "Western Slavic marker") and 17 Y-STRs on 154 DNA samples from Ukrainians. These results were compared to previously published data from Slavic and non-Slavic populations. Mitochondrial DNA control-region sequences of about 2700 samples obtained from Eastern (Russians and Ukrainians) and Western Slavs (Czechs, Poles and Slovaks) were used to select 51 samples representing mitochondrial H5 haplogroup. For these mtDNAs the entire genome sequences were determined. Together with published data we have collected 210 complete mtDNA sequences belonging to the H5 haplogroup. Thus, improvement of the resolution of H5 haplogroup phylogeny and evolutionary age estimation of H5 subhaplogroups were possible. We were able to identify a number of new subhaplogroups (i.e. H5a1a1, H5a1r, H5a1s and others) as well as to show that the founder H5 clade (12-15 ky old) is mainly represented by individuals from southern Europe. We also showed some subclusters (H5a1a, H5a1f, H5e1a, H5a2 and H5u), which are mainly represented by residents of central and eastern Europe. The evolutionary age of these subhaplogorups was dated between 2-5 ky. The overall picture of Y chromosome and mtDNA diversity in Central Europe corresponds well with origin and later expansion of Corded Ware European culture. Thus, we suggest that genetic continuity existed in Central Europe between Bronze Age and Middle Ages when the earliest Slavic tribes were described.

Y-chromosomal STR analysis in the Pashtun population of Southern Afghanistan

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Afghanistan is a landlocked country in the heart of Asia and since the dawn of humankind Afghanistan has faced centuries of turmoil, strife, conflict, warfare, distress, social unrest, difficult climate, harsh terrain and due to its unique geostrategic position in Eurasia which has historically attracted commerce and conflict. It is an important stop along the Silk Road, connecting the far eastern civilizations to the western world. A 5000-year history of constant invasion. Afghanistan has been repeatedly invaded andconquered by rulers and super powers, neighboring interference in this conflict-tattered land for centuries yet rarely leading to the conquest of this rugged and challenging terrain nation. Afghans are not only shepherds, farmers and nomads but also intense fighters and fierce warriors. Currently very limited genetic studies have been performed in Afghan populations. 17 Y chromosomal short tandem repeats (Y-STRs) were analyzed in 125 unrelated Pashtun (in hindi:Pathan) males residing in the Kandahar region of Southern Afghanistan. A total of 92 unique haplotypes were observed. The predominant haplotype reached a frequency of 9.6%. The haplotype diversity was 0.987 and the discrimination capacity 73.6%. Analysis of molecular variance (AMOVA) reveals a considerable regional stratification within the country as well as between different Pashtun (Pathan) groups from Afghanistan, Pakistan and India.

Y-chromosomal phylogeny of Porja and Savara lineages in Andhra Pradesh, India

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DNA Polymorphisms reveals a population's genetic structure, migration and admixture in the past, susceptibility to illness and genetic causes of diseases. Human Genome, DNA sequencing offers an evidentiary alternative fossil based prehistoric reconstructions about origin and evolution of human beings. The uniparental inherited non-recombining haploid mtDNA and Y chromosome became an extremely powerful tool in phylogenetic studies. Studies on mtDNA, Y chromosome and many autosomal regions supports that modern humans originated in Africa between 166,000 and 249,000 years ago then expanded throughout Africa and into rest of the world with little or no interbreeding between modern humans and archaic populations lived elsewhere in the old-world, including the Neanderthals in Europe and Homoerectus in Asia. In the absence of selection, genetic drift and founder effects have played a major role in shaping haplotype frequencies, giving rise to haplogroups and sub haplogroups that are often restricted to specific geographic areas and/or population groups. Y chromosome haplogroups can trace back paternal lineages into the past. Genetic data on Indian populations reveals that Indian caste and tribal populations share a common ancestry in India. Indian Y-chromosomal data consistently suggest a largely South Asian origin for Indian caste communities and therefore argue against any major influx, from regions north and west of India. Hence present paper is mainly focusing on genotyping for human Y-chromosome to investigate paternal lineages among two primitive tribal populations in Andhra Pradesh, India. We have analyzed Y-Chromosome SNPs of the two primitive tribal groups of the Austro-Asiatic linguistic families, Savara from Vizianagaram district and the Porja from Visakhapatnam district of Andhra Pradesh, India and compared the data with other relevant data of Indian tribal populations. The results reveals the occurrence of the O2a* haplogroup 68% and 61% in Savara and Porja population respectively. Further we found H1a*, H*, C*, R1a1*, R2, P*, O2a*, O3a3C*, and F* haplogroups in smaller frequency. From the MDS plot we found both Savara and Porja are closely associated with Asur, Korku, Birja, Bhumji, Pando and Khasi tribal populations of India. The results suggests that the Savara and the Porja, Austro-Asiatic language speaking tribes of Andhra Pradesh, represent a genetic continuity between the populations of North East India and South East Asia, thereby advocating that East India could have been a major corridor for the movement of Austro-Asiatic populations.

Migration distance rather than migration rate explains genetic diversity in patrilocal groups

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In patrilocal groups females preferentially move to join their mate's paternal relatives. The gender-biased gene flow generated by this cultural practice is expected to affect genetic diversity across human populations. Greater female than male migration is predicted to result in a larger decrease in between group differentiation for mitochondrial DNA (mtDNA) than for the non-recombining part of the Y chromosome (NRY). We address the question of how patrilocality affects the distribution of genetic variation in human populations controlling for confounding factors such as ethno-linguistic heterogeneity and geographic distance which possibly explain the contradictory results observed in previous studies. By combining genetic and bio-demographic data from Lesotho (Southern Africa) and Spain (Europe), we show that preferential female migration over short distances in patrilocal communities appears to minimize the impact of a general higher female than male migration rate, suggesting patrilocality might influence genetic variation only at short ranges.

Rapidly mutating Y-chromosomal STRs

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Y-chromosomal STRs (Y-STRs) currently applied in forensic analysis provide high but not maximal resolution in male lineage differentiation and their power to separate male relatives is low. Consequently, using current marker sets, conclusions from Y-STR analysis usually cannot be made on the individual level as anticipated in the forensic arena, which represents a major draw back. However, it would be desirable to combine the advantage of Y-chromosome analysis in separating male from female DNA components in mixed stain analysis, such as it is essential for solving cases of sexual assault, with the advantage of autosomal STR analysis in individual identification, which often is not informative in mixed stain analysis. In order to find rapidly mutating Y-STRs for differentiating male relatives, we previously performed a large mutation rate study analyzing 186 Y-STRs in ~2000 father-son pairs and identified a set of 13 markers that mutate considerably faster than all other loci tested. We recently showed that this set of rapidly mutating (RM) Y-STRs can differentiate male relatives in a large number of cases (i.e., 67% of relatives separated by 1-20 generations whereas Yfiler only did 15%). Furthermore, we showed that RM Y-STRs increase male lineage differentiation considerably (i.e., by 8% from 90.4% with Yfiler to 98.3% with RM Y-STRs in the worldwide HGDP-CEPH samples). To provide further prerequisites for forensic applications of RM Y-STRs, and to make the data finally available for haplotype search in future forensic case work, we now carried out a multicenter study involving 70 institutions from around the world. We collected quality-controlled data for the 13 previously identified RM Y-STRs from over 10,000 unrelated male individuals; for about 7000 of them conventional Y-STR data (Yfiler) are available to us for comparative analysis. Furthermore, we collected RM Y-STR data on >1000 father-son pairs for additional male relative differentiation testing. In this plenary talk I will summarize the benefits of using RM Y-STRs in forensic analyses and will provide the first results and conclusions from this multicenter study on behalf of the RM Y-STR Study Group[a].

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Development criteria for a next generation Y-STR multiplex for forensic applications

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The downstream application of a forensic Y-STR multiplex defines the criteria used for developing a kit that is fit for purpose. If the objective is to exclude close patrilineal relatives of the suspect then markers with a high mutation rate might prove beneficial. On the other hand, in kinship analysis markers with high mutation rates might prove problematic. The inclusion of currently used Y-STR markers in the next generation multiplex should be considered given that the existing Y-STR databases are already populated with profiles containing this information. New markers could be added to enhance the capabilities of already existing Y-STR multiplexes. These enhancements could combine features such as (1) mini-STRs, (2) the inclusion of highly discriminating markers which could allow for better differentiation of paternal lineages in populations with low Y-chromosome diversity and (3) rapidly mutating markers. A next generation kit should also improve the overall balance, resistance to inhibitors of the PCR and provide shorter time to results. This presentation will discuss a strategy for the development of an enhanced Y-STR multiplex that combines well-known loci as well as recently characterized, highly discriminating Y-STRs into one single reaction. This multiplex will enable higher discrimination in paternal lineages, combined with unprecedented capabilities in terms of robustness, sensitivity and male specificity to recover information from forensic samples.

The PowerPlex® Y23 System: a single system for casework and database (direct amplification) Y-STR analysis

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Y-STR testing is an established tool in the forensic casework, paternity testing and genealogy communities. The PowerPlex® Y23 System combines the seventeen Y-STR loci in current commercially available Y-STR kits (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA-H4) with six new highly discriminating Y-STR loci (DYS481, DYS533, DYS549, DYS570, DYS576, DYS643). The additional loci and increased gene diversity increases scientists' ability to distinguish individuals from different paternal lines from one another, enabling more meaningful analyses. The PowerPlex® Y23 system is a robust multiplex highly tolerant of many amplification inhibitors, including hematin, humic acid, and tannic acid. The system has proven sensitivity, detecting minimal amounts of male DNA in the presence of excessive amounts of female DNA. Complete Y-STR profiles are detected with 62pg of male DNA in the presence of 400ng female DNA (6450-fold excess). This Y-STR system is an excellent tool for testing in sexual assault cases in which sperm was not detected from intimate swabs collected from female victims. The PowerPlex® Y23 system also supports direct amplification from a variety of substrates, including blood and buccal samples on FTA® paper (GE Healthcare/Whatman) and other commonly used paper substrates. This system produces reliable Y-STR profiles from swabs when processed with Promega's SwabSolution™ Kit. Additionally, Y-STR amplification with this system provides significant time savings due to a reduced cycling time of 90 minutes--less than half the time required for other available Y-STR kits. By offering superior inhibitor tolerance, proven sensitivity, and protocols for both casework and databasing, the PowerPlex® Y23 System sets a new standard for Y-STR analysis.

Y chromosome specific nested PCR pre-amplification method for improved detection of male DNA

Hanson E[1,*], Solivan M[1], Strauss S[2], Di Pasquale F[2], Engel H[2], Ballantyne J[1,3], presented by Scherer M

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Male DNA-containing samples collected from sexual assault or homicide victimscan contain very low levels of cellular male DNA admixed with a large number of femaleepithelial cells. This often results in failure to obtain an autosomal STR typing from themale DNA donor. Y-STR analysis can be used to overcome this problem. However thereare still many instances where such an approach does not work. This is particularly sowhen an intimate sample is collected many days after the incident, usually as a result ofdelayed reporting by a rape victim or when there is a significant time interval betweendeath and recovery of a rape/homicide victim's body or when the samples manifestsome degree of degradation. Recent technological advances in the area of DNA profilingoffer the opportunity to improve the number of specimens that can be successfullyanalyzed. Therefore it may be possible to develop strategies to overcome the problemsassociated with low levels of male DNA in a background of female DNA. We havedeveloped such a method using a selective amplification of Y chromosomal genomicDNA prior to standard Y- STR analysis. This 'genomic partitioning' appears to be aneffective strategy to further increase the signal to noise ratio of the Y chromosomal DNAcompared with the epithelial DNA and hence allow clear unambiguous male profiles tobe obtained. Additionally, such an approach could also be used to improve the analysisof touch or contact DNA samples which often contain small amounts of male DNA.In this work, we have developed a 17-locus Y chromosome specific nested PCRpre-amplification multiplex and have performed an initial validation to demonstrate itspotential suitability for use with forensic samples. The pre-amplification takes less than2 hours to perform and can be used in conjunction with commercially available Y-STRamplification kits. The use of the nested PCR pre-amplification prior to Y-STR analysisallows for the recovery of Y-STR profiles from as little as 5 pg of male DNA (~ 1 diploidcell) from various body fluids and tissue (blood, semen, saliva and skin). No interferencefrom female DNA was observed even in the presence of female DNA in 100,000-foldexcess. We have demonstrated the method's ability to recover Y-STR profiles fromtouch and contact DNA samples as well as extended interval post coital samples (> 5days after intercourse).

Mt-GoNL: deep sequencing of 750+ complete Dutch mtDNA genomes

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The Genome of the Netherlands (GoNL) is a national collaboration aimed at establishing a map of Dutch genetic variation by whole genome sequencing of 250 Dutch trio families. This trio-based setup and the high mtDNA coverage (on average 1100 times) in each sample give us the unique opportunity to study both population-wide and intra-human variation on the mitochondrial genome. We developed a number of techniques in which mtDNA can assist in quality control of whole genome sequencing experiments. The high coverage facilitates simple detection of contamination with a low percentage of foreign DNA. One such case is present in our data set and its contamination has been confirmed by autosome analysis. Analysing violations of the inheritance patterns allowed identification of sample swaps. One of the goals of our mtDNA study is to define the Dutch mtDNA phylogenetic tree. Preliminary results show that the data set contains more than 165 different mtDNA haplogroups, where H and its subclades are most abundant, representing about 40% of individuals. This is in concordance with previous studies on the distribution of haplogroup H in Europe. 127 haplogroups are observed in at least 2 individuals and 68 mtDNA haplogroups are present among 4 or more individuals. In some of our samples we noted a disagreement with respect to the defining polymorphisms for some haplogroups, indicating opportunities for refinement of the mtDNA phylogenetic tree. Finally, indepth analyses, ongoing at time of abstract submission, are carried out to search for previously not recognized mtDNA variants including those defining new mtDNA haplogroups. This abstract is submitted on behalf of the Genome of the Netherlands (GoNL) Consortium (http://www.nlgenome.com).

Applications of Personal Genome Machine (PGM™) in SNP-based human identification

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Genomic degradation and deconvolution of complex mixtures are among the many challenges present in DNA-based human identification. Single nucleotide polymorphisms (SNPs) have attributes that make them valuable identifiers for human forensics, providing a extensive pool of possible markers and a platform for small amplicon PCR-based detection. Although SNPs are for the most part bi-allelic, it has been estimated that approximately fifty polymorphic SNPs are necessary to complement the discriminatory power of the 13 core STR loci [1]. High-throughput technologies for SNP genotyping such as microarrays and next-generation sequencing have yielded a wealth of population data, and subsets of these markers have been isolated for identification [1, 2, 3]. The PGM™ 314 chip was used to sequence a panel of fifty unlinked SNPs with high heterozygosity and low fixation indices (Fst) curated by the SNP for ID consortium [1]. Genomic DNA was extracted from five control individuals. Polymorphic loci were amplified using the 50-plex primer pool, barcoded, pooled, and sequenced on a single chip. SNP genotypes were called upon mapping reads to a reference genome. Larger SNP panels targeting known and well-characterized polymorphic regions have also been constructed using the Ampliseq Designer, a tool for creating custom primer pools for non-conflicting multiplex PCR, used to yield the template for PGM™ library prep. Future directions include panels of phenotypic SNPs for forensic applications. 1. Sanchez et al. A multiplex assay with 52 single nucleotide polymorphisms for human identification. Electrophoresis (2006) vol. 27 pp. 1713-1724 2. Pakstis et al. SNPs for a universal individual identification panel. Human Genetics (2010) vol. 127 pp. 315-324 3. Kidd et al. Developing a SNP panel for forensic identification of individuals. Forensic Science International (2006) vol. 164 pp. 20-32

Next Generation MtGenome Sequencing for forensic purposes using the Ion Torrent PGM

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Sequencing mitochondrial genomes (mtGenomes) provides valuable information in population, medical and forensic genetics. The emergence of next generation sequencing (NGS) technologies has greatly increased sample through-put compared to traditional Sanger-type sequencing but most platforms are cost-intensive and require long hands-on times. Also, the scientific community has witnessed increased error rates in first attempts, which is a sensitive issue in forensics.We have evaluated the Ion Torrent Personal Genome Sequencer (PGM, Life Technologies, Carlsbad, CA, USA) for the generation of mtGenomes for a couple of reasons: a) it is easy-to-use compared to other NGS instruments, b) the level of automation is relatively high, c) the costs of the instruments and chemistry required are comparatively cheap and d) the application of barcodes allows the simultaneous sequence analysis of mtDNA (or other loci) of multiple individuals. We are currently testing the performance of the PGM by sequencing mtGenomes of samples that were already typed using conventional Sanger-type sequencing. This allows direct comparison of data derived from both technologies and evaluation of the PGM for forensic purposes.

The impact of PCR and DNA sequencing artifacts in 454 LifeSciences data on the interpretation of mtDNA heteroplasmy

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The use of second generation DNA sequencing approaches to interpret mtDNA heteroplasmy and mixtures is of great interest to the forensic community. While successful approaches have been developed using different platforms and covering different portions of the mtDNA genome, the impact of PCR and sequencing-based artifacts on the interpretation process is significant, and the extent of the impact is still relatively unknown. Until the community has a better understanding of the impact, and the issues are addressed appropriately, the full use of this technology will be limited. Our laboratory has focused on amplicon-based sequencing of the mtDNA control region. For example, the HV1 segment was previously analyzed using the 454 GS Junior instrument from Roche, and mock mixtures were generated to evaluate the system's ability to reliably detect low level heteroplasmy (Holland et al, Croatian Medical Journal, 52:299, 2011). Mock heteroplasmic variants were detected routinely down to a component ratio of 1:250 (20 minor variant copies with a coverage rate of 5,000 sequences), and were readily detected down to 1:1000 (0.1%) with expanded coverage. The analysis of 30 individuals, representing 25 different mtDNA haplotypes, revealed a rate of reportable heteroplasmy that was ~10 times that of conventional Sanger sequencing; 44% versus 4%. However, considerable PCR and sequencing-based artifacts (including errors) were also detected in the data, and in almost all cases, chimeric sequences were observed. Interpretation of heteroplasmy is not impacted to a great extent by the presence of these artifacts, while the deconvolution of mtDNA mixtures is greatly impacted given the increased number of nucleotide differences between the two (or more) components. We will present our analysis of the PCR and sequencing-based artifacts observed in 454 data, collected from hundreds of thousands of data points, and how these artifacts impact the interpretation of mtDNA heteroplasmy and mixtures.

Weighing the differences: the application of Mass-Spectrometry for mtDNA control region analysis

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The first applications of mass spectrometry (MS) techniques for DNA analysis were developed in the late 1990s. Soft electrospray ionisation allows the analysis of relatively large molecules, such as DNA fragments in the size range of 150 base pairs. One distinctive feature of DNA typing by mass spectrometry is that analysing PCR fragments yields so-called base composition profiles (BCPs), which enumerate the base counts for each of the four nucleotides, but do not contain any base-specific positional information. Consequently, BCPs are somewhat less informative than conventionally sequenced mtDNA haplotypes, which, in turn, results in a loss of discrimination power in the forensic context. However, two important advantages compensate for this limitation. First, mtDNA analyses has never found broad application in the forensic field, as Sanger-type sequencing is laborious, prone to error and the laboratory handling involves numerous steps that harbour the inherent risk of sample mix-up and contamination. The here presented MS-approach with the PLEX-ID instrument (Abbott) is highly automated by support of integrated liquid handling, sample integrity is achieved by barcode-assisted processes and BCPs are generated and interpretable by dedicated user-friendly software that opens the field of forensic mtDNA analysis also to non-expert laboratories. Second, sample through-put is generally low for sequencing approaches, especially when degraded mtDNA is under investigation as many individual fragments need to be amplified, sequenced and interpreted. With PLEX-ID this complexity is greatly reduced and analyses times are decreased from days to hours, which would allow a laboratory to answer court requests in a timely fashion.

Interpretation of lineage markers

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The interpretation of Y-STR and mtDNA forensic profiles continues to be challenged by the dependencies among profile elements, because of a lack of recombination, and by relatively small databases. We can address the database size issue to some extent by using tagging SNPs in large publicly-available datasets, and we also use these datasets to demonstrate the independence of autosomal, Y-STR and mtDNA profiles. The dependencies among lineage profile elements suggests concentrating on the profile as a unit, and invoking the population genetic arguments of Ewens whereby increasing numbers of profile elements, and consequent increasing mutation rates, leads to decreasing match probabilities. The somewhat different approaches of Brenner (2010) and Buckleton, Krawzcak and Weir (2011) are examined with reference to actual and simulated data.

Evidentiary strength of a rare haplotype match

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When a rare haplotype is shared between suspect and crime scene, how strong is the evidence linking the two? The relevant number is the conditional probability of an innocent person to match the crime scene profile, given available data including the crime scene profile, population data, and scientific knowledge.

The traditional methods of evaluating the strength of DNA evidence include several institutional misconceptions. First and most fundamental is to confuse probability (a summary of available data) with population frequency (which is not available). Next, the normal statistical assumption that sample frequency reasonably estimates population frequency ignores among other things scientific knowledge and fails badly for rare traits. Third is forgetting to condition on the crime scene observation. Hence the traditional paradigm in forensic practice is to confuse sample frequency with population frequency and in turn confuse that with probability. The consequence is very roundabout and unsound reasoning.

More direct methods are not difficult. One simple and validated idea is based on the proportion singletons – of haplotypes that occur just once – in a sample, which I call κ (kappa). For present-day Y-STR typing, the most common situation by far is that the crime scene haplotype is previously unseen in a sample of size n-1. In that case the matching probability for an innocent suspect is $(1-\kappa)/n$. Even simpler is to count the rate of pairwise matches in a reference database. This empirical matching probability is appropriate on average hence slightly conservative for previously unseen types.

Estimating trace-suspect match probabilities for singleton Y-STRs haplotypes using coalescence theory

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An important task of statistical forensic genetics is to evaluate the evidential weight provided by some genetic data of interest, and the most consistent (and therefore generally recommended) way of doing so is by means of the likelihood ratio. One particularly important match probability in this context is the probability that a certain individual (the ”suspect” in a crime case) has the same DNA profile as another individual drawn at random from the same population (the donor of a trace found at the crime scene). Methods to estimate this trace-suspect match probability are well established for autosomal STRs, with most of them assuming statistical independence between the markers included in the profile.

Use of lineage markers, such as Y-chromosomal short tandem repeats (Y-STRs) or mtDNA polymorphisms, has several advantages over autosomal markers in forensic practise, for example, when solving cases of sexual assault. However, due to the lack of recombination, and therefore statistical independence between loci, the calculation of match probabilities is more challenging for lineage than for autosomal markers. In particular, when considering Y-STR haplotypes usually comprising 7 to 17 loci, the proportion of singletons (i.e. haplotypes observed only once) in a reference database may become so large that traditional count estimates of the corresponding match probabilities are rendered unsatisfactory.

In this talk we present how to estimate trace-suspect match probabilities using coalescence theory and demonstrate that it performs well in comparison with other estimators (such as the ”haplotype surveying” method and Brenner's kappa method) based on a simulation study.

Because the coalescent-based estimator is rather computational-intensive, we shortly discuss the practical applicability of this method.

Y-STR mutations: what paternity cases can tell us about the relationship between allele length and mutation rate

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Forensic applications of Y-STR markers often require estimates of the respective mutation rates. This is especially true for phylogenetic analyses, where mutation rates must be estimated to calibrate the molecular clock. Usually the locus-specific fraction of observed mutations in a sample is used for these purposes. However, it is well known that the rate of STR mutation not only varies across loci, but does also depend on the allele length for any given locus, although the exact nature of this dependency is still unclear. We describe some simple STR mutation models, including a novel logistic one, incorporating allele length as a factor [1]. To fit and compare these models, data on the inheritance of Y-STRs in father-son duos, accumulated from the forensic literature, were used. For each locus and each model, we employed a maximum likelihood approach to estimate the model parameters. For most loci considered, a certain version of the logistic mutation model was found to provide the best fit according to Akaike's Information Criterion. This implies that the mutation probability at these loci increases non-linearly with allele length at a rate that differs between upward and downward mutations. [1] Jochens A, Caliebe A, Roesler U, Krawczak M. Genetics 189(4): 1403-1411, 2011.

Testing Y chromosome STR mutation rates using deep-rooting pedigrees

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Y-chromosome STR mutation rates play an important role in forensic sciences, especially for determining the paternity probability during paternity testing. Mutation rate estimates for genealogical approaches (like father-son pair typing) normally give a higher mutation rate estimate than population based studies with a known recent population history. The Afrikaner population serves as a good system to test the y-chromosome STR mutation rate with a deep-rooting pedigree approach. Deep-rooting pedigree studies are also useful for estimating the non-paternity rate of a population. South African founding fathers from Europe often introduced unique surnames into the Afrikaner population from the mid 1600s to 1700s. South Africa has an active genealogical research society and records often permit researching an individuals' pedigree that stretch back to the founding father. Y-chromosome STR mutation and non-paternity rates were estimated from 20 deep-rooting pedigrees with 6545 meiotic transfers. Subjects that are genealogically connected to the founding fathers were typed for 17 y-chromosome STR loci with the AmpliflSTR® Yfiler® kit (Applied Biosystems). A recent surname (Greeff) based study estimated an average STR mutation rate of 4.85×10^{-3} based on a single old South African family. We estimated an average STR mutation rate of 3.1×10^{-3} and when we combined our data with the Greeff study, we obtained an average STR mutation rate of 3.5×10^{-3} . Our mutation rate estimate is higher than the father-son pair approach (2.8×10^{-3}), but still falls within the confidence interval limits we obtained. We estimated a non-paternity rate of 0.51% for the Afrikaner population, which is even lower than the recent estimate of 0.78% for the Afrikaner population.

Analysis of mutation rates in purported brother pairs with 17 Y-STR loci

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Y-STR mutation rates have most commonly been computed between father and son pairings. For missing persons casework and examination of skeletonized human remains, reference materials may not be available from the father or son of the decedent. While mitochondrial DNA (mtDNA) analysis is frequently used, in cases of commingled remains where one or more individuals share a mitotype, additional genetic means of identification need to be utilized. The Armed Forces DNA Identification Laboratory (AFDIL) has been examining the use of Y-STR analysis as an addition to the toolkit of genetic analysis. In the course of validating the use of the AmpFLSTR® Yfiler® PCR Amplification Kit (Life Technologies) with skeletonized human remains, the need to examine mutation rates in the presence of increased generational steps was identified. Four hundred seventy-six individuals were analyzed to determine the mutation rates at 17 Y-STR loci. The individuals selected were self-reported brothers to missing individuals and also had at least one other brother available for comparison. Reference materials were obtained in the course of family reference collections for mtDNA analysis. Sibling indices (both full and half) were determined using the AmpFLSTR® Identifiler® Kit (Life Technologies) for each pair. Of the 194 alleged sibling pairs successfully amplified, the majority of individuals were found to be consistent with their purported brother. However, twenty of these pairs varied from each other by one or two loci, each by a single repeat. Of note is the finding of thirteen cases of questionable genetic relationship, including possible non-paternity and non-sibship.

Searching for dependencies between mitochondrial and Y chromosome DNA markers

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Combining and updating evidences when conducting reasoning with uncertainty is a key topic in forensic evidence interpretation. When dealing with identification of human remains or paternity investigation there is a great need to use different kinds of genetic markers. Attempts to evaluate the combined effect of separate items of evidence may be complicated without the knowledge about possible dependency between the evidences. Autosomal DNA profile proportion in the population is formed by the product rule and the mtDNA and Y-chromosome haplotypes estimates are the counts in a database. Hence multiplying them is the first estimate of the joint probability on the hypothesis on identity. However, possible dependencies may occur between the patterns of population distribution among genetic markers with different modes of inheritance. The aim of the study was searching for the presence of pairwise linkage disequilibrium between mtDNA and Y-chromosome haplogroups distribution due to possible South Poland population admixture. Both mtDNA and Y-chromosome haplogroups were assigned for 305 reference unrelated male samples. There were observed 31 different mitochondrial and 17 Y-chromosome haplogroups, respectively, and 88 combined haplotypes (average gene diversity over two markers = 0.708412). Performed exact test using Markov chain of pairwise linkage disequilibrium indicated that there were no linkage in heritability between these markers (exact p-value = 0.903564). Previously performed population study of autosomal STR loci, showed that the F_{ST} value for South Poland population was negligible. Hence, the real structure of South Poland population, as checked by frequencies of particular autosomal, mitochondrial and Y-chromosome markers, respectively, demonstrates high degree of differentiation without of significant disturbing population processes. The results provide acceptable arguments for multiplying the likelihood ratios obtained from different kinds of genetic markers without any further assumptions. This conclusion is very important for routine forensic casework when assessing the combined value of genetic evidence is necessary.

Mine, yours, ours? Sharing data on human genetic variation

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The achievement of a robust, effective and responsible form of data sharing is currently regarded as a priority for biological and bio-medical research. However, it has been argued that its possible advantages in terms of better exploitation of data and optimized use of resources may be counteracted by the time and economic costs required, by underlying ethical concerns, and conflicts of interest with patenting discoveries. In this contrasting scenario, empirical evaluations of data sharing may be regarded as an indispensable first step in the identification of critical aspects and the development of strategies aimed at increasing availability of research data for the scientific community as a whole. Research concerning human genetic variation represents a potential forerunner in the establishment of widespread sharing of primary datasets. However, no specific analysis has been conducted to date in order to ascertain whether the sharing of primary datasets is common-practice in this research field. To this aim, we analyzed a total of 543 mitochondrial and Y chromosomal datasets reported in 508 papers indexed in the Pubmed database from 2008 to 2011. A substantial portion of datasets (21.9%) was found to have been withheld, while neither strong editorial policies nor high impact factor proved to be effective in increasing the sharing rate beyond the current figure of 80.5%. Disaggregating datasets for research fields, we could observe a substantially lower sharing in medical than evolutionary and forensic genetics, more evident for whole mtDNA sequences (15.0% vs 99.6%). The low rate of positive responses to e-mail requests sent to corresponding authors of withheld datasets (28.6%) suggests that sharing should be regarded as a prerequisite for final paper acceptance, while making authors deposit their results in open online databases which provide data quality control seems to provide the best-practice standard. Finally, we estimated that 29.8% to 32.9% of total resources are used to generate withheld datasets, implying that an important portion of research funding does not produce shared knowledge. By making the scientific community and the public aware of this important aspect, we may help popularize a more effective culture of data sharing.

Posters

mtDNA haplogroup F1a'c is a genetic risk factor for nasopharyngeal carcinoma in Chaoshanese

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Mitochondrial DNA (mtDNA) is susceptible to oxidative damage and harbours a high rate of mutation. It is clear that mitochondial play a role in cancer development. We conducted a case-control study to investigate the possible association between mtDNA haplogroups and nasopharyngeal carcinoma (NPC) in 201 NPC patients and 201 normal controls from Chaoshan population. Binary logistic regression analysis with adjustment for gender and age showed that mtDNA haplogroup F1a'c was associated with a significant risk of NPC (P = 0.040, OR = 2.589, 95% CI: 1.045 - 6.412). A separate comparison of gender or age further confirmed that F1a'c was a risk factor to NPC in males or in patients with age ≥ 40 years old (P = 0.009, OR = 6.697, 95% CI: 1.619-27.706; P = 0.015, OR = 4.099, 95% CI: 1.323-12.703, respectively). However, no such significant correlations were found in females or in subjects younger than 40 years old. The analysis stratified by gender and age further revealed that the frequency of F1a'c was significantly higher in NPC patients than in controls for men ≥ 40 years (P = 0.015, OR=8.250, 95% CI: 1.498-45.429). In summary, our study showed that mtDNA haplogroup F1a'c tended to have an increased risk for NPC in Chaoshanese.

Mitochondrial DNA polymorphism and matrilineal genetic composition of Chaoshan population in China

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Chaoshanese, a Chinese population residing in Chaoshan region in southern China, is an admixture population whose gene pool is derived from the Central China Han and southern aboriginal natives, as determined by our previous autosomal short tandem repeat (STR) study. To determine the matrilineal genetic composition of this population, we investigated the mitochondrial DNA (mtDNA) of various Chinese populations and analyzed the genetic relationship between Chaoshanese and other Chinese populations from the perspective of maternal inheritance. mtDNA polymorphisms in the hypervariable segment regions (HVS-I and HVS-II) and the COII/tRNA^{Lys} intergenic region were typed in 201 Chaoshan individuals. The mtDNA HVS-I sequence and haplogroup frequency data of other Chinese populations were collected and used for population comparison. Population relationships were examined by principal component, multidimensional scaling and median-joining network analyses. In addition, admixture analysis was performed to estimate relative contribution of northern Hans and southern natives to the Chaoshan population. Our results showed that the Chaoshanese, along with other southern Hans, is well separated from the northern Hans and occupies an intermediate position between northern Hans and southern natives. In matrilineal gene pool of the Chaoshan population, genetic composition of southern natives and northern Hans accounts for about 50%, respectively, indicating that the matrilineal genetic composition of Chaoshan population consists of both northern Han and southern native origin.

Insights into South-American colonization through mtDNA analysis in native Colombian populations

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Aiming to add some clues on the colonization of the American continent, more precisely the entrance points and dispersion routes taken, studies with lineage DNA markers such as mitochondrial DNA (mtDNA) and Y chromosome have been performed, which allow tracing back the history of populations because they are transmitted without recombination to the descendants.

In the present study we determined the matrilineal ancestry of samples from two regions in Colombia through mtDNA analysis. Based on the observation of Native American haplogroups in both populations, we also intended to perceive if there are differences that could indicate different migrations towards the South of the continent.

The complete mtDNA control region was sequenced for 98 samples from the two groups (38 Emberá from Antioquia and 60 samples from various ethnic groups from Cauca) and compared with the revised Cambridge Reference Sequence. Haplogroup frequencies were calculated and phylogenetic analyses were performed.

The vast majority of haplogroups found in both Colombian populations are typically Native American. Our results show that while in the Antioquia region, the Emberá population presents a very reduced number of haplotypes, all belonging to haplogroups A, B and D, the Cauca region is more diverse and has a significant percentage of C haplogroup lineages. When dividing the Cauca group into smaller speaking groups it is visible that they are obviously distinct and behave as small populations that have suffered evolutionary forces along time such as genetic drift and bottlenecks. When comparing with other populations from literature, there is a notable proximity between Chibchan speaking groups, whereas non-Chibchan remain differentiated. Regarding a geographic separation, there is no visible substructure. Instead, distinct patterns are visible both in northern and southern populations within Colombia which may result from distinct ancient routes.

Comparative mitochondrial DNA analyses in Myanmar and the distinct genetic position of the Karen people within this multi-ethnic population

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Background: Myanmar (Burma) is the largest country in Southeast Asia, with a population of 56 million people subdivided into more than 100 ethnic groups. The Bamar represent the largest group (68%) amongst them. Ruled by changing kingdoms and dynasties and lying on the trade route between India and China, Burma was influenced by a variety of cultures. Since Burma's independence after the British occupation, minorities suffer from government's repression and especially the Karen people (7%) struggle against the domination of the Bamar culture. We analyzed the mtDNA control region of 327 unrelated donors from Myanmar according to highest quality standards. To refine haplogroup information, 44 selected lineages were subjected to complete mitochondrial genome sequencing.Mitochondrial data from Myanmar were compared with other Southeast and East Asian populations.

Results: The distribution of the macro-haplogroups M and N and the geographic assignment of the haplogroups were typical for Southeast Asia, however, the frequency of individual haplotypes was very specific in Myanmar.In general, the Myanmar sample exhibited pronounced mtDNA diversity, with the ethnic group of the Bamar being the most diverse. The haplotype composition of the Karen people, in contrast, was significantly more homogenous than other ethnic groups. We found 10 new mtDNA lineages, represented by 15 haplotypes, mostly within macro-haplogroup M. No traces of European contribution to the gene pool were detected.

Conclusions: The multi-ethnic population and the complex history of Myanmar are well reflected in its distinct mtDNA heterogeneity, nevertheless genetic diversity cannot exclusively be attributed to variation due to ethnicity. In this region with its long history of human settlement, plenty of mitochondrial haplogroups, especially in the complex haplogroup M, await to be newly described.

Multiplex mutagenically separated PCR assays for simple and rapid screening of East Asian mtDNA haplogroups on forensic samples

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Nucleotide polymorphisms in human mitochondria DNA (mtDNA) have been one of the main issues in population genetics, clinical medicine, and forensic science. Especially, determination of human mtDNA haplogroup has become a useful tool to study human evolutionary history and to infer the matrilineal bio-geographic ancestry. In forensic field, the screening of mtDNA haplogroups by genotyping of mtDNA single nucleotide polymorphisms (SNPs) can help guarantee the quality of mtDNA sequence data as well as can reduce the need to sequence samples that do not match. Here, a multiplex mutagenically separated (MS) PCR system was developed for simultaneous rapid detection of 14 coding region SNPs and one deletion motif representing common mtDNA haplogroups of East Asia; mtDNA haplogroups M, G, D, D4, D5, M7, M8, M9, M10, N, A, N9, R, F, and B. As the D4 haplotypes occur most frequently (> 25% in Koreans), additional multiplex MS PCR system was also developed for the determination of four coding region SNPs to further define D4 subhaplogroups D4a, D4b, D4e, and D4j. The multiplex MS PCR system we developed has the advantage of being a one step procedure that requires only a single PCR amplification with allele-specific primers and allowing straightforward designation of haplogroups along the branches of the phylogenetic tree. Therefore, it would be a simple, rapid, and reliable detection method useful for large-scale screening of mtDNA variations to determine East Asian mtDNA haplogroups.

Mitochondrial DNA data of five Philippine Negrito populations

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Understanding human history, ancestry and human origins is a source of identity and pride for a country and its people. A multicenter population genetic study involving 73 Asian populations (which includes five Philippine "Negrito" groups) and 54,794 autosomal single nucleotide polymorphisms (aSNPs) showed that Austronesian language speaking Filipinos are genetically associated with other Austronesian groups in Asia and that ancestors of Filipino "Negrito" groups were part of the first wave of modern humans that populated the Asia-Pacific region (HUGO Pan-Asian SNP Consortium 2009). This new discovery underscores the importance of characterizing lineage genetic markers found on the mitochondrial DNA and Y-chromosome in order to provide a greater understanding of the genetic variations that appear to be specific for some Philippine groups. This study therefore aimed at investigating the patterns of mtDNA variation in the five Philippine "Negrito" groups included in the multicenter study and comparing these with previously generated mtDNA sequence of Filipinos in Philippine regional centers (National Capital Region, Cebu City and Zamboanga City) (Tabbada et. al., 2009) and other Asian countries in order to evaluate existing theories of prehistoric migration and peopling of the Asia-Pacific region. Archived samples consisting of FTA®-bound buccal DNA from sixty (n=60) unrelated individuals belonging to five Philippine "Negrito" groups namely, Aetas of Zambales, Aetas of Bataan, Agtas of Bicol, Irayas of Mindoro and Atis of Panay Island, were amplified at mtDNA HVRI and HVRII and sequenced using Big Dye® Terminator chemistry (Applied Biosystems). Mitochondrial DNA haplogroup affiliation of each sample was determined using mtDNA tree Build 10 (http://www.phylotree.org). If needed, the haplogroup identity of some samples was confirmed using PCR-RFLP reaction. Classification of Philippine "Negrito" mtDNA sequences and a comparison with existing Philippine and Asian populations' mtDNA data will be presented.

An approach to Equatorial Guinea demographic genetic history through maternal lineage analysis

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Equatorial Guinea is located in the Central-West African coast, limited by Cameroon on the North and Gabon on the South. The country is composed by continental and an insular portion, this one formed by several islands, among which highlight Bioko where the capital Malabo is located. Due to its geographical situation, just behind to the start point of the Bantu expansion, the majority of the country populations belongs to this group, been the Fang tribe the most representative, encompassing 80% of the total inhabitants. The main goal of this work was to assess the pattern of diversity in maternal lineages from Equatorial Guinea population. For this propose sequences of the complete control region of mitochondrial DNA (16024-576bp) in 50 unrelated individuals were obtained, through the analysis of a 3.5 Kpb mitochondrial segment resulting from the amplification of two shorter overlapping fragments. The majority of the haplogroups detected are characteristic of Sub-saharan populations. In this sense, haplogroups L0, L1, L2 and L3 represent 92% of the total maternal lineages, whereas previous Y-chromosome results showed a lower proportion of male lineages of sub-Sahara African ancestry, due to the presence of European lineages that explained around 14% of the total variability. Gene diversity level found (0.9984 ± 0.0044)was in the same range of the value revealed analysing the Y-chromosome polymorphisms (0.9994 ± 0.0019). The number of different mtDNA haplotypes detected, 48 out of 50, which bring to light the great diversity hallmark of African populations. Nevertheless, the diversity in Equatorial Guinea appears too high for a population strongly influenced by the Bantu expansion. This kind of studies are crucial to get a full understanding of the origin and history of human populations, although more analyses are still ongoing, such as comparisons with surrounding populations located also in the Western African coast.

Mitochondrial DNA data of Cabo Verde immigrant population living in Lisboa

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Mitochondrial DNA (mtDNA) analysis found an important role in forensic genetics, especially when nuclear DNA analysis does not give a conclusive response. It is a powerful tool to exclude samples as originating from the same matriline. Features that increase the vested interest of mtDNA are the high copy number per cell, maternal inheritance, absence of recombination, and high mutation rate. Due to higher overall mutation rate, control region is comparatively enriched in sequence variation and therefore its analysis is important to establish haplotypes and haplogroups. Haplogroup assignment became noteworthy to clarify the history and demographic past of a population. As well as occurs all over Europe, in Portugal, and particularly in Lisboa, immigrant populations are increasing. The Instituto Nacional de Medicina Legal e Ciências Forenses is carrying out a comprehensive genetic study with the aim of portray the genetic diversity of the immigrants who live in Lisboa. Within that objective the present study intends to: obtain the mtDNA variability of Cabo Verde Immigrant Population Living in Lisboa and classify haplotypes into haplogroups. MtDNA control region was amplified using two pairs of primers L15997/ H016 and L16555/ H599. The cycle sequencing was performed using the ABI Prism® BigDye® Terminator v.3.1 Cycle Sequence Kit (Applied Biosystems, Foster City, CA) and BetterBuffer (Microzone Limited, Sussek, UK). Analysis was done with ABI DNA Sequencing Analysis V5.2 and SeqScape v2.5. The obtained haplotypes were compared with the Cambridge Reference Sequence (CRS) and typed following the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC). Haplogroups were determined on the mtDNAmanager. Preliminary results showed great variability, with high frequency of unique haplotypes and significant values of nucleotide and sequence diversity. The majority of mtDNA sequences were included into specific African mtDNA haplogroups and a minority of mtDNA lineages belongs to West Eurasian haplogroups.

Dissection of mitochondrial DNA haplogroup L3b and its forensic applications

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In a study of the variability of mitochondrial DNA (mtDNA) control regions HVI/II in a population sample (n=100) from Ivory Coast (North West Africa), a random match probability (RMP) of 1.56% was observed. When mtDNA haplotypes were affiliated to haplogroups, based on patterns of shared haplogroup-associated polymorphisms, it could be seen that the most common haplotype, observed with a frequency of 6%, could be assigned to haplogroup L3b. In general, haplogroup L3b (representing 16% of all mtDNA lineages) showed a low haplotype diversity (RMP=20.31%) in the Ivorian population sample.

Here we describe the combination of two multiplex single base extension assays, interrogating a total of 13 mtDNA coding region single nucleotide polymorphisms (SNPs), for the dissection of mtDNA haplogroup L3b. By multiplex SNP typing, the assignement of putative L3b mtDNA sequences was confirmed on a molecular basis, and further discrimination among samples sharing identical HVI/II haplotypes was possible. Individuals carrying the most common haplotype could be subdivided in L3b2 (n=4) and L3b1a (n=2) and, on the whole, the RMP was reduced to 1.38%.

The described assays can therefore increase the forensic informativeness of mtDNA analysis in investigations involving subjects of West African descent, allowing rapid screening of samples in high volume casework and exclusion of multiple suspects.

Refined characterization of Portuguese mtDNA variability for forensic purposes

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The analysis of mtDNA polymorphisms has become a useful tool in fields such as forensic science, human population genetics and molecular evolution studies. The amount of data accumulated from different populations all over the world is massive. Still, the Portuguese population is poorly characterized for the mtDNA complete control region and lacks a comprehensive database of good quality sequences.

For this reason we have sequenced the mtDNA complete control region of 298 unrelated individuals equally distributed through North, Center and South of Portugal and undertaken haplogroup classification. Specific coding region single nucleotide polymorphisms (SNPs) were further genotyped by single base extension multiplex reactions in individuals classified within haplogroup H, which encompasses over 40% of the total mtDNA variation in Western Europe.

A total of 106 studied individuals (35.6%) were classified within haplogroup H. The southern region showed a slightly lower percentage of individuals classified in haplogroup H (32%) in comparison with the other two regions (37%). The remaining sequences were classified into the following major haplogroups: R* (incl. B, J, T - 20.1%), U (incl. K - 19.5%), N* (incl. I, W, X – 10.1%), R0 (except H – 7.0%), L (except L3 – 5.4%) and M (incl. D - 0,7%) (Nomenclature according to PhyloTree.org - mtDNA tree Build 14, 5 Apr 2012).

With this study, we provide a better characterization of the mtDNA variability in the Portuguese population by collaborating and contributing to the enrichment of the EMPOP database (www.empop.org). Finally, by comparing the three regions, North, Center and South, we will understand whether the Portuguese sample should be considered as a whole, when applied to routine forensic genetic casework.

MtDNA haplogroup distribution in Finland

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According to earlier studies the diversity of mtDNA haplotypes within Finnish population is comparable to other European populations and, geographically relatively uniformly distributed. This is in stark contrast with Y-chromosomal haplotypes showing considerable differences between East and West Finland.

To complement the previous haplotype-level analyses, we have in the present study investigated the Finnish mtDNA diversity on subhaplogroup levels. In order to gain more in-depth view on the Finnish population history we explored differences in regional distribution and diversity of haplogroups and subhaplogroups. The haplogroup information was inferred from control region HV1 and HV2 data using mtDNA tree Build 14 (PhyloTree.org, 5 Apr 2012). The sample consisted of 384 haplotypes (303 when rapidly mutating sites 309.1C(C), 315.1C, 16182C, 16183C and 16193.1C(C) were omitted) from 832 randomly chosen individuals geographically assigned to different regions of Finland based on donors' place of residence.

Despite the mtDNA diversity in Finland is, compared to Y-chromosomal variation, high and relatively uniformly distributed, the haplogroup data showed some statistically significant geographical differences within Finland. By contrasting these haplogroup diversity patterns with other markers, data inferred for different ancestral source populations and possible past colonization routes, a more detailed picture of the Finnish past can be achieved.

Optimization and validation of a mitochondrial DNA assay in a German population sample and application to highly degraded DNA

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Mitochondrial DNA is a powerful tool in forensic casework. The high copy number of mitochondrial genomes permits a successful amplification even then if nuclear DNA is widely degraded or limited. Due to the maternal inheritance mitochondrial DNA analysis make it possible to reconstruct relationships and to trace back branches of haplogroups and their subgroups to conclude chronological and geographical details of migration. Recently, Parson and colleagues developed and evaluated three different mitochondrial DNA genotyping assays for the analysis of the whole mitochondrial DNA control region, dependent on the quality of the DNA (Parson & Bandelt, 2007: mitochondrial standard assay; Berger & Parson, 2009: Midi-Mito assay; Eichmann & Parson, 2008: Mini-Mito assay). In our study we present a German population study of about 200 individuals aiming to optimize the handling of the assay. Sequencing and evaluation were performed according to EMPOP quality studies. Our study presents a summary of our experiences and difficulties which we obtained during the establishment and validation of the two published mitochondrial analysis methods (standard assay and Midi-Mito assay) in our routine work. Besides the application of the mitochondrial DNA method to population genetics we also show the applicability of this assay in our practical casework. We used the optimized Midi-Mito assay to determine the haplotypes and their associated haplogroups of skeletons for which no or only few STR genotyping information was available. Because of the high sensitivity of the mtDNA assay profiles could be generated from picograms of DNA and highly degraded DNA.

MtDNA analysis of Mocoví population, southern most Guaycurú speakers in South America

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Mocoví is the south most ethnicity, linguistically associated to Pilagá, Toba and Wichi tribes, representing the Mataco-Guaycurú speakers group. Mocoví inhabit Chaco and Santa Fe provinces and amounts over 15.000 people. Aiming to increase the knowledge of Guaycurú speakers, Mocoví individuals (N= 27) and additional Tobas (N= 47) from Santa Fe province were analyzed by means mitochondrial DNA Control Region sequencing under EMPOP guidelines. The results were compared with previously obtained sequence data of three ethnic groups that inhabit the area of Argentinean Gran Chaco: Pilagá (N=55, from Formosa province), Toba (N=64), from Formosa and Chaco provinces and Wichi (N= 48) from Formosa. The entire mtDNA Control Region (16024 to 576) was sequenced in a total of 241 unrelated individuals. Sequencing strategy included the use of at least six primers for each sample in order to obtain unambiguous sequences. The four Native American haplogroups (Hgs) were present in these groups, with diverse frequencies. Hgs B2 and D (subhaplogroups D1 and D4g) are well represented in Pilagá, Toba and Wichi. Mocoví showed high frequency in HgA (52%) meanwhile the frequency of HgD is very low (7%). Haplogroup C is absent in Tobas and Wichi, whereas is present in Pilagá and Mocoví with a frequency around of 17%. The haplotype diversity in Mocoví sample was higher than the rest of the groups and highest genetic distances were observed when this group was compared with Toba, Pilagá and Wichi. Genetic distances were all significant, except between Toba´s groups. This work allowed us to analyze the spread of mitochondrial lineages from Mataco-Guaycurú speakers and to find the relationship between the individuals that inhabit nowadays the Argentinean Gran Chaco.

A very high level of Native American ancestry in an Amazon, Brazil, urban population inferred by mitochondrial DNA and indels markers analysis

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The Brazilian population is highly heterogeneous as a result of five centuries of interethnic mating between Native Americans, European colonizers and Africans brought to the country during 3 centuries of slavery. This study aimed to assess the proportions of interethnic admixture in the urban population of Santa Izabel do Rio Negro, Amazonas, Brazil, whose inhabitants are known as descendants from Tukano Oriental and Aruak groups, using mitochondrial DNA (mtDNA) and autosomal Ancestry Informative Markers (AIMs). A total of 100 individuals were characterized by sequencing analysis of the full mtDNA control region.The haplogroups A, B, C and D that characterize the mitochondrial amerindian lineages were present in very high frequencies as about of 39%, 21%, 28% and 11%, respectively, while only one sample showed european ancestry. The same population sample had been genotyped for 46 AIM-Indels markers and the ancestry estimates were then assessed using HGDP-CEPH samples as ancestral reference. The global admixture estimates showed a predominantly Native American ancestry (83,5 %) followed by European (12,0 %) and African (4,6 %) contributions.. The interethnic admixture scenery captured by autosomal AIM-Indels and mtDNA genetic information at Santa Izabel do Rio Negro are in agreement with historical records.

Mitochondrial control region sequences of the Czech Republic population and a comparison to other populations

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The correct use of mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate population databases to determine the relative rarity of the haplotype of the tested sample. The aim of this study was to evaluate the results of full HVRI and HVRII mtDNA sequences of more than 250 unrelated individuals and to compare the data to the previously published Czech population data obtained using PCR-RFLP. The Genetic diversity (GD) and Random match probability (RMP) of the Czech mtDNA population data were also compared to the other European populations. The results indicate that the full control region sequencing can bring more precise population information that is useful for the comparison with other data sets and also for the forensic identification purposes.

Insertion/delection polymorphisms in South Portugal Caucasian population: a preliminary study

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Human genetic identification is usually based on the study of STR markers. Recent advances in forensic genetics have focused on the development of genotyping assays using shorter amplicons in order to improve the successful amplification of degraded samples. Single Nucleotide Polymorphisms (SNP) and Insertion/Deletion polymorphisms (INDEL) have in this kind of forensic samples, considerable potential in the field of identification, since they can combine desirable characteristics of both, STR and SNP. In this study, a set of 30 biallelic Delection/Insertion polymorphisms (DIP or INDEL) distributed over 19 autosomes plus Amelogenin in a single multiplex PCR reaction was applied to 100 healthy and unrelated caucasian individuals (50 males and 50 females) selected from our laboratory casework samples. DNA was isolated from blood stain cells by chelex method and DNA concentrations were estimated by Real Time PCR using the Quantifiler™ Human DNA quantification kit on an ABI Prism 7500 (Applied Biosystems). INDEL's amplification was performed with Investigator DIPplex® kit (Qiagen) in an ABI Prism 3130xl, according to manufacturer's instructions. Allele distribution, Observed (OH) and Expected Heterozigoty (EH), and Hardy Weinberg (HWE) departure were estimated by Arlequin 3.5.1.2. Preliminary results reveal that at the population level the overall loci meet HWE ($p<0.05$) and so, in the near future, these markers will be an important tool in our routine genetic identification.

Internal validation of the AmpFlSTR Yfiler amplification kit and calculation of the genetic diversity of 17 Y-short tandem repeats in the Norwegian population

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A total of 290 haplotypes were identified using seventeen short tandem repeats included in the AmpFlSTR Yfiler amplification kit. 264 (91%) of the haplotypes were unique and the overall haplotype diversity was 0.997. Average locus diversity was 0.63. As a part of the internal validation the stutter, N-banding and pull ups were calculated and were found to correspond to Mulero et al. (2006). After implementation of the kit, the interlocus balance (rfu values) has been found to be greater for the stains than reference samples.

Y chromosome diversity in the South and East Kazakhstan

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In the past, the territory of Kazakhstan has been inhabited by different nomadic tribes. Since then, its territory has always been a keystone on the geopolitical map of the Central Asia. 17 Y-specific STR loci have been analyzed in a total of 166 unrelated males from two different regions in Kazakhstan in order to understand the genetic structure of the present day Kazakh population represented in a current study by South Kazakhstan region (n=99) and East Kazakhstan region (n=67). A questionnaire was filled by participants to confirm their ancestry up to the last three generations. Sample collection, questionnaire and informed consent used in this study were approved by an appropriate ethical committee. Multiplex PCR amplification of 17 loci was performed using AmpFℓSTR® Yfiler™ kit (Applied Biosystems). The genotyping data have been deposited to the Y-Chromosome Haplotype Reference Database (accession numbers YA003700, YA003729, www.yhrd.com). A total of 92 different Y-STR haplotypes were observed in the studied Kazakh population sample. Statistical analysis of data was carried out using Arlequin ver. 3.5.1.2 Overall haplotype diversity was 0.930 and discrimination capacity was 0.076. The commonest haplotype 15-12-29-23-10-13-12-13,18-10-12-15-19-15-17-19-12 (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a,b, DYS438, DYS439, DYS437, DYS448, DYS456, DYS458, DYS635, GATA H4) was shared by 41 individuals from East Kazakhstan region. The second most prevalent haplotype 16-13-29-25-10-11-13-12,13-10-10-14-22-15-17-21-11 was shared by 13 individuals from South Kazakhstan region. Not a single haplotype was found to be common between South and East Kazakhstan providing evidence for population substructuring. Absence of recent common ancestors in these two subpopulations is also supported by a specific historical source of information common for a few Central Asian countries, called “Shezhire”, which is a verbal and sometimes written genealogical data kept in the families and passed down from father to son.

Genetic structure of the Y chromosome does not suggest intraregional sex-biased dispersals in the population of Asturias (Northern Spain)

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Traditional cultural and anthropological studies of the autochtonous population of Asturias have indicated a deep-rooted custom of matrilocality, dated at least to the pre-Roman “Astur” tribes. This custom is deemed to have important influences in the present-day distribution of social and linguistic features inside this Spanish autonomous community. A previous survey found a significant genetic structure for the mitochondrial DNA control region between different regions of Asturias, indirectly supporting this theory. In this study, we performed Y-Chromosome genotyping in 184 males with at least two generations of Asturian paternal ancestry, using 14 SNP markers and 13 STRs. Haplotype data and place of birth of the paternal grandfathers of all the volunteers were used in a SAMOVA analysis, which created maximally-differenced population groups. Final between-group variation was significant and almost reached 5%, with a group composition that was similar (but not equal) to that found for the mitochondrial DNA. BARRIER and MIGRATE analyses were also performed to obtain male gene-flow tendencies between these groups. This structure and its associated features are discussed in the light of historical and genetic data, finding that sex-biased social customs cannot be invoked as important driving forces behind the present day patterns of the Asturian gene pool.

Y-STR haplotypes and the genetic structure of Pathan populations in FATA and NWFP of Pakistan

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The Pathans represent the tribes who speak Pashto and inhabit mainly the North West Frontier Province (N.W.F.P.), adjoining tribal areas of Pakistan, and southern and eastern parts of Afghanistan. Pathans are the second-largest ethnic group in Pakistan, and have reigned as the dominant ethnic group in Afghanistan for over 300 years. Here, 22 Y-STRs were analyzed in 270 unrelated Pathans from N.W.F.P. and Federally Administered Tribal Areas (FATA) of Pakistan; 234 are from N.W.F.P. and 36 are from FATA. In haplotype analysis, 200 different haplotypes were observed with haplotype diversity of 0.9957 in Pathans from N.W.F.P. and 32 different haplotypes were observed with haplotype diversity of 0.9889 in Pathans from FATA. In analysis of molecular variance between the neighboring Pathan populations inhabiting Afghanistan, Pakistan and India, the Pathan population from N.W.F.P. did not show significant difference from Pathans of North Afghanistan and Yousafzai Pathans of Khyber Pakhtunkhwa, Pakistan. Interestingly, the Pathan population from FATA did not show significant difference from Afridi Pathans in India. Considering that the Afridi Pathans of Pakistan mainly inhabit rough hilly area covering most of the Khyber Agency, FR Peshawar and FR Kohat in FATA, the close genetic distance between Afridi Pathans in India and the Pathan population from FATA of the present study and their large genetic distance from other Pathan populations reveal a considerable regional stratification between different Pathan population groups from Afghanistan, Pakistan and India but high homogeneity between Pathan populations sharing the history of inhabiting the same region.

Development of six-SNPs assay for forensic analysis in European population

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Y-chromosomal SNPs analysis show regional specificity useful in forensic investigation for inferring the male genetic background of individuals and population and to predict biogeographical origin of the donor of a crime scene sample. Due to its exclusively paternal inheritance, the Y-chromosome has been extensively used in evolutionary and forensic genetics to investigate the phylogeny and the history of population and their migration. A large scale parsimonious phylogenetic tree representing worldwide Y-chromosome variation has been constructed and comprises major haplogroups. The aim of this study was to set-up six multiplex assays based on SNaPshot kit to identify markers inside major clades of European population. Specifically, we design PCR and minisequencing primers targeting a total of 33 Y-mutations downstream R1*, I*, J2*, G* and E1b1b1* haplogroups. The PCR fragments were chosen to get the shortest product possible in order to improve the performance in degraded samples (amplicons principally ranging from 56 bp to 140 bp). This assay based on a 6-multiplex PCR reaction is a suitable tool for detecting the main European haplogroups in forensic casework and population study.

Genetic journey of the N1c haplogroup

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Binary and Y-STR polymorphisms associated with the NRY region of the human Y chromosome preserve the paternal genetic legacy that has persisted to the present, permitting inference of human evolution, population migration and demographic history.The NRY region of the Y chromosome acts much like mtDNA to reveal the structure among human populations and possibly to infer the order and timing of their descents. In the present study, we have investigated the origin of haplogroup N1c-Tat phylogeographic structure and the genetic relationship of Eurasian populations by examining STR variation in a large number of individuals. We have identified 54 samples as the haplogroup N1c-Tat from 5 population groups (N=632). To place the results into a wider geographic context, we included 209 samples from published sources and 296 samples from the FTDNA public database into the phylogenetic analysis. According to previous studies haplogroup N-M231 is of East Asian ancestry. Our results suggest that N1c-Tat mutation probably originated in South Siberia 8-9 thousand years ago and had spread through the Urals into the European part of present-day Russia. Its distribution is not fully correlated with the spread of Uralic languages. Turkic-speaking ethnic groups in South Siberia have high N1c-Tat presence and STR variance, while the N1c-L550 subgroup largely occurs among non-Uralic-speaking European populations. Only the European N1c-Tat (xL550) subgroup can be linked to the spread of Finno-Ugric languages from the Kama-Urals area ~6,000 years ago. The subgroup N1c-L550 cannot be considered Finno-Ugric origin and its carriers might have been assimilated by Indo-European groups, resulting in their spread across Europe in historical times with Vikings and Balto-Slavs. Based on the present study Buryats were dominated by a young, about 800-years old N1c-Tat cluster, which suggest that this ethnic group could be a relatively recent admixture of Mongolian conquerors with a Paleo-Siberian population groups.

Phylogeographic analysis of human Y chromosome diversity in eastern Africa

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Encompassing an area characterized by enormous geographic variety, as well as ethnic, linguistic and cultural diversity, eastern Africa has seen remarkable levels of human migration and interaction over a very long period of time. Despite its importance for the evolutionary history of our species, this region has nonetheless seen less evolutionary genetic research than other regions in the African continent. In a study of 750 males from 25 eastern African populations, we have analyzed 107 Y-specific biallelic polymorphic markers, many of which here described for the first time. We observed 44 different Y chromosome haplogroups, some of which - haplogroups A-M13/V3, J-M267/V44, E-M215 and E-M329 - showed peculiar and interesting geographic distributions in the region. Phylogeographic analysis of the data showed that the gene pool of eastern Africa has been shaped by different processes associated with the physical geography of the area, social structure of some populations, demic diffusions and important cultural innovations.

Y-chromosome variation in geographically and linguistically isolated populations from oriental Alps

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As a result of ancient and complex peopling processes and the presence of physical barriers, the Alpine area provides unique opportunities for anthropological and genetic studies of linguistic and geographical isolation. In this study, we have investigated the genetic structure of thirteen populations (for a total of 533 individuals) from the Eastern Italian Alps. These include six linguistically isolated groups - five German-speaking communities (among which Cimbrians from Luserna and Giazza, and the communities from Sappada, Sauris and Timau) and one Ladin-speaking group from Trentino. All samples were typed for 17 microsatellites (Y-filer profile) and 57 Single Nucleotide Polymorphisms in order to get an exhaustive overview of their Y chromosome variation and haplogroups composition. Such results were compared with genetic data available for European populations, with the aim of investigating the effect of linguistic and geographic isolating factors on the genetic structure of the populations under study. Finally, we carried out a comparative analysis between Y-chromosomal and mitochondrial data on the same Alpine populations in order to test sex biased genetic patterns possibly related to cultural factors.

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Haplotype analysis of 23 polymorphic Y-STR markers in Northwest of Argentina

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Catamarca and Jujuy are two provinces of Argentina, placed in the extreme Northwest of the country, close to the borders with Chile, and Bolivia. Both of them are included in NOA (Northwest Argentina), one of the six historical-geographical regions that divide Argentina. Different studies on South American populations have proposed that actual population is composed by Native American maternal lineages and European paternal lineages with different proportions of them among regions. 23 Y-STRs were studied in samples from autochthonous individuals from Jujuy (n=59) and Catamarca (n=27), using PCR primers and conditions described previously. To assign the most probable haplogroup of each individual, we used Haplogroup Predictor program that assigns the most probable haplogroup from the Y-STR profiles. All samples of the 2 populations show different haplotypes for 23 Y-STRs, except two males from Jujuy. The total haplotype diversity was estimated at 0.9915 in Catamarca and 0.9959 in Jujuy according to minimal haplotype. Results about inferred haplogroups showed that the population sample from Jujuy was mainly composed by Native American haplogroup Q (49.15%), while the population sample from Catamarca was mainly composed by European haplogroups (77.78%), mostly R1b. Haplogroups distribution in Jujuy province, as some other populations from NOA, was different from most of the populations outside NW of Argentina. The rest of provinces of Argentina show higher frequencies in non Native American lineages, such as R1b or E, than in Native American lineage, varying from one locality to another. Jujuy province shows a composition of haplogroups of closer proximity to neighboring countries, such as Bolivia, which could suggest a common origin or/and a continued gene flow.

Y chromosome DNA variation monitored in Slovakian populations by SNP and STR analysis

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In the Slovak Republic live approximately 400.000 Romanies. The highest concentration of the Romany population lives in Eastern Slovakia. Slovakian Romany population represents relatively isolated ethnic group providing a unique opportunity for genetic study. This study provides additional population genetic data of the three different East Slovakian populations. Many Y-chromosomal single nucleotide polymorphisms (SNPs) are now available. The haplogroups which they define are highly non-randomly distributed among populations. 49 Y-chromosomal single nucleotide polymorphisms (SNPs) and 17 Y-chromosomal STR loci were tested in 525 unrelated male individuals from East Slovakia: Slovak population (n=243) and two Romany populations: Romany group from region Spiš (n=125) and Romanies from region Prešov (n=157). DNA was isolated from the buccal swabs using JetQuick kit according to the manufacturer's protocol. Samples of DNA were amplified with the AmpFlSTR Y filer PCR Amplification Kit and analyzed with Genetic Analyzer 3500 (Applied Biosystems,USA).The Y-SNP loci were tested with the amplification of 10–12 ng genomic DNA, performed in ABI 7500 systems using TaqMan probes. Haplogroup diversity values were calculated and the populations were compared with G-test. The Slovakian Y chromosomal haplogroups were R1a1-M198, R1b1-P25. In the Romany population from Prešov it was haplogroup H1a-M82 and R1a1-M198. In Romany group from Spiš the most frequent haplogroup was J2a2-M67. The H1a-M82 haplogroup was the most frequent in both Romany groups with the frequency as high as 50%. The Romany populations were significantly different in comparison with Slovakian population. This work is the result of the implementation of the project ITMS 26220120041. **Keywords:**Y-chromosome; SNPs; STR, Romanies, Slovakia

Y chromosome diversity in Piedmont

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Y-chromosomal variability of 17 short tandem repeat (STR) and 18 single nucleotide polymorphisms (SNP) loci was evaluated in three different population samples from Piedmont (North West Italy): Biella (Northern Piedmont, n=80); Trino (Central Piedmont, n=46); Cuneo (Southern Piedmont, n=90). Participating individuals were carefully selected based on their genealogical ancestry: Biella and Cuneo samples included adult males with at least three generations of residence (thus predating the industrial immigration from Southern Italy that took place in the 1950s); the Trino sample consisted of subjects belonging to an association dating back to the Middle-Ages, which limits membership to families who have been settled in the village since the 13th century.

AMOVA analysis of Y-SNP haplogroups indicated a variation among individuals within and among sampling areas of 99.97 and 0.03% ($p=0.378\pm0.013$), respectively. Trino showed reduced values of Y-STR haplotype diversity ($h=0.983$), compared to Cuneo ($h=0.999$) and Biella ($h=0.998$). Absence of significant variation among sampling areas (0.10%, $p=0.350\pm0.004$) was confirmed by AMOVA at haplotype level, andno significant differences were observed when pairwise genetic distances (R_{ST}) between the three samples were calculated. On the contrary, multiple significant R_{ST} values were obtained when Piedmont samples were compared with available population data from North East, Central and South Italy.

The obtained results confirm that, although the Y-chromosomal landscape of Piedmont seems fairly homogeneous, genetic heterogeneity is present in Italy at the inter regional level.

Genetic analysis of 17 Y-STR loci in Pashtun population from Swat Valley, Pakistan

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17 Y-Chromosomal short tandem repeats (Y-STRs) included in the AmpFlSTR Yfiler amplification kit (Applied Biosystems, Foster City, USA) DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and Y-GATA-H4 were analyzed in 71 unrelated Pashtun (Pathan) males residing in the Swat Valley of Khyber Pakhtunkhwa province, Pakistan. A total of 43 unique haplotypes were observed. The predominant haplotype reached a frequency of 23.94%. The haplotype diversity was 0.860465 and the discrimination capacity 60.56%. Analysis of molecular variance (AMOVA) reveals a considerable regional stratification within the country as well as between different Pashtun (Pathan) groups living in Pakistan.

Novel Y chromosome polymorphisms in Native American haplogroup Q1a3a1

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Since 1996, when DYS199 marker was first described on the Y chromosome (Underhill et al), Native American populations have been characterized by DYS199/M3 polymorphism. The high frequency of its derived state, attaining 77% in Native American males (Bortolini et al. 2003), might be explained by: (a) an extremely reduced number of colonists carrying this marker or (b) increased reproductive fitness. Y-chromosome characteristics related to male fertility may influence the high prevalence of haplogroup Q1a3a1 within Native Americans. Therefore, the aim of this work was to analyze STS markers on the Y chromosome linked to fertility in samples from confirmed fathers belonging to haplogroup (hg) Q1a3a1 and reference hg R1b1a2. Unrelated male samples from routine paternity casework have been analyzed including those belonging either to hg Q1a3a1 or R1b1a2. Paternity was assessed by PowerPlex®16 and Y-chromosome haplogroups by Real Time PCR followed by HRM. Afterwards, sY1261, sY1191, sY1291, sY1206 and sY1201 STS markers located in AZFb/c regions have been amplified and analyzed by capillary electrophoresis. A novel SNP variant was found on the distal copy of the sY1206 marker showing a G>T variation exclusively in haplogroup Q1a3a1. Moreover, marker sY1291 showed a 21bp size difference between haplogroup Q1a3a1 (517pb) and R1b1a2 (538pb) due to a homopolymeric T track. This last finding is in concordance with previously published results (Lin et al. 2006), where a length difference was first characterized for this marker but not linked to any haplogroup or population. Nevertheless, no differential deletions of the Y-STSs markers analyzed were found between haplogroup Q1a3a1 and R1b1a2, conversely to what has been previously described (Repping et al. 2004, 2006). These results were consistent within all the analyzed samples. The present results describe two novel Y chromosome polymorphisms and disprove the presence of the b2/b3 deletion as a characteristic of hg Q1a3a1.

Population data for 8 Y-chromosomal STRs (not included in Y-filer™ kit) in a population sample of Czech Republic

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The newly designed Y-chromosome miniSTR pentaplexes I and II include 8 “non-core“ Y-STR loci DYS388, DYS426, DYS444, DYS446, DYS447, DYS449, DYS459, DYS481 plus additional 2 Y-STR loci DYS392 and DYS438 that overlap with the of Y-filer™ kit. The amplicon sizes were designed as “miniSTRs” so the pentaplexes can be also used for degraded and ancient DNA typing. The Y-pentaplexes I and II were used to obtain the allele frequencies and gene diversities for the population sample of more than 140 unrelated individuals from the Czech Republic.The data show that the additional Y-STR loci (on top of Y-filer) are extremely useful not only in the complex genealogical studies but also as a research tool for the Y-chromosome and surname correlation studies.

Y-STR haplotype diversity of a native population of Cabo Verde living in Lisboa

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Cabo Verde is an African archipelago located in the West African coast of the Atlantic Ocean. Historically, native population of Cabo Verde is the result of an admixture of Caucasian European colonizers and African slaves. As well as it occurs all over Europe, in Portugal and particularly in Lisboa, immigrant populations are clearly increasing. According to Portuguese Foreign Affair Services, in 2010 the number of immigrants from Cabo Verde living in Lisbon was up to 34234. The Y-chromosome, male specific and constitutively haploid, is one of the smallest human chromosomes with an average size of 60 million base pairs that largely escapes to meiotic recombination. The combinations of allelic variants of markers along the chromosome, defined as haplotypes, pass intact from generation to generation. They change only by mutation and so preserve a record of their history. This unique biology has led to the widespread use of genetic markers in determining patrilineal relationships and haplogroups within and between populations, with application in population studies and provides a powerful discrimination tool for routine forensic applications. The main goal of this study is to complement our previous studies with autosomal STR, X-chromosome and mitochondrial DNA of genetic structure of Cabo Verde immigrants, with patrilineal/Y-haplotype characterization. We studied a sample of 50 unrelated and healthy male individual's natives of Cabo Verde, actually living in Lisboa and undergoing forensic investigations in Portuguese Instituto Nacional de Medicina Legal e Ciências Forenses. We analysed the markers included in the European Minimal Haplotype (EMH): DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, and DYS393, recommended by the International Y-STR User Group for Identity Testing, plus the DYS437, DYS438 and DYS439, recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and also DYS448, DYS456, DYS458, DYS635 e GATA H4, to increase haplotype and gene diversity.

Y-chromosomal STRs mutation analysis by the PowerPlex® Y23 system

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Y-STRs analysis is a useful tool in forensic casework especially for kinship testing as well as in genealogical research for inference on population history and evolution. The new commercial kit PowerPlex® Y23 system (Promega) allows for the detection of seventeen commonly used Y-STR loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635, Y-GATA-H4) plus six new highly discriminating Y-STR loci (DYS481, DYS533, DYS549, DYS570, DYS576, DYS643) increasing the ability to distinguish individuals from different male lineages and providing more meaningful analyses. In this study, we analyzed the 23 Y-STR markers of the PowerPlex® Y23 system in a particular deficiency paternity case, previously typed with a different commercial kit, which showed single repeat mutations at DYS439 and DYS385. In addition, we investigated a total of 60 male germline transmissions of confirmed paternity cases (probability > 99.9%) reporting preliminary data about mutation rates of the six new Y-STR loci. Knowledge about mutation rates and the mutational process of Y-chromosomal STRs is crucial for the correct interpretation of resulting genetic profiles and for improving forensic probability calculations. This study increases Y-chromosome haplotypes data and provide a contribution to the Y-STR mutation databases as requested by the forensic community.

Determination of the Y chromosome DNA haplotype and mtDNA haplotype from 50 males from 17 countries

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To obtain reference DNA necessary to identify the ethnicity of males, we compared the detection rate of haplotype Y chromosome DNA of 50 men from 17 countries. Additionally, mtDNA haplotypes were determined and they were considered as the reference for maternal ethnic identification. In low diversity Y-STR loci (DYS389 I, DYS391, DYS439, DYS392, H4, DYS437) weak differences in allele patterns were present among the three ethnic groupings. However, the DYS385ab locus displayed high diversity of allele types and allele pattern differences varied with ethnicity. Within locus DYS390 allele 21 was absent from European, rare in Asian and predominate in African samples. Utilizing ISOGG classifications it was possible to determine Y-haplogroup from pre-determined Y-SNP ratios. In the future, urban crime, large natural disasters and accidents have the possibility of increasing as globalization progresses. In such ethnically mixed urban areas it will be increasingly necessary to determine the ethnic origin of individuals. In these cases it is necessary to perform quick individual identification through DNA typing. For this to take place, in addition to the establishing of a methodology, further collection of DNA polymorphism information from each ethnic group is necessary.

Forensic efficiency of combined Y/mt profiles in seven Iranian groups

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Despite cultural and geographic barriers, the genetic landscape of Iran as defined by Y-STR and mt-HVSI markers is considered fairly homogeneous. Hence, low LR values for both, individual and ethnic assignment of the DNA evidence, may be obtained. In the present research, haplotypes at Y-filer and HVSI panels of loci were analyzed in 130 healthy unrelated males from seven Iranian native groups. A separate analysis of the haplotype profiles failed to detect a population sub-structure whereas the outlying position of three ethnic groups (Balochs, Qashqaee and Zoroastrians) as well as maximum levels of genetic diversity and discrimination capacity (H, DC=1) were obtained in every group by combining the two profiles.It can be argued that, when combined, the forensic efficiency of routinely used panels of loci can be largely sufficient to resolve cases of human identification even in genetically homogeneous populations.

Ancestry evaluation of Rio de Janeiro population by screening the Y chromosome and mitochondrial DNA

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The Brazilian population is derived from an admixture between Native Americans, Europeans, mainly Portuguese, and Africans, which are mainly bantu speakers that had been brought to the country as slaves between the XVI and XIX centuries. The historical records lack information concerning the regions of Africa from where the slaves had been taken to America. The analysis of 17 Y-STR loci in 152 healthy self declared afro-descendants revealed 151 different haplotypes with eleven of them showing typical bantu founder profiles. The haplotypic diversity was very high (0.9988/+-0.0003), revealing no strong founder effects. Additionally, 43 Y-SNP markers were typed in the same samples allowing the discrimination of 15 haplogroups. A significant proportion of European lineages (71%) were detected, followed by African (26%) and Amerindian (3%) lineages. African chromosomes were mostly represented by E1b1a-M2 and E1b1a7-M191 chromosomes that are the most frequent ones in all Bantu groups, including those in Central Africa. The haplotypes from samples carrying typical African haplogroups were compared with those found in several bantu populations. Pairwise F_{ST} showed no significant genetic distance among the self declared African descendants from Rio de Janeiro and Angola populations (F_{ST} = 0.06131 , p= 0.00069 ± 0.0003). Out of 152 samples, 70 were sequenced for the entire mtDNA control region in order to provide maternal ancestry information. The mtDNA data showed a very important African contribution (81%), represented mainly by the L0, L1, L2 and L3 haplogroups, followed by Amerindian (14%), represented by the haplogroups A and C, and a lower number of European lineages (4%). In summary, concerning the Rio de Janeiro population, the Y-chromosome lineages of self declared afrodescendants, show a major contribution of Europeans, followed by Africans and, to a lesser extent Amerindians, as we have already described for the general population. On the other hand, data from mtDNA revealed to be the African, followed by the Amerindian, the mainly maternal inheritance sources contributing to both samples from general population and self declared afro-descendants from Rio de Janeiro population, while a poor European lineages contribution has been observed.

Haploid markers in DNA identification process in Croatia

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Identification process in Croatia involves DNA based identification since the method was recognized as the precise and straightforward way to answer the question of identity. The majority of identification cases are those of war victims' remains. As the time goes by, DNA analysis is taking over the most important role in the identification process because bodies exhumed twenty years after the death could hardly be identified by any other method. For many reasons, such as cost effectiveness and informative results, typing of nuclear STR markers using different multiplex kits is the first choice. It has been almost 15 years since we introduced haploid DNA markers as a method of choice for typing samples to help identification of skeletal remains. In some cases, even when genomic DNA was successfully amplified, the additional information was still needed for final conclusion. Y chromosome STR multiplex kits are especially useful in cases when only male relatives are available for testing. When the genomic DNA is present in a low copy number, it is severely degraded or only distant relatives are available, we sequence two hypervariable segments (HV1 and HV2) within the mitochondrial non-coding region. Here we will present several identification cases where haploid typing results were very helpful in establishing the identity of the human remains.

Forensic science applied on prehistoric remains - a nine fold burial of the 4th millennium BC raised questions about kinship, locality, and circumstances of death

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A monumental earth construction of the Salzmünde culture (3400-3025 BC) with 165 burials was discovered during excavations at the eponymous site Salzmünde in Saxony-Anhalt, Germany. Especially a multiple burial with potsherd filling that contains four adult women and five subadults raised questions about kinship, locality and cause of death. This exceptional ninefold burial is the matter of a transdisciplinary and integrative project combining archaeological, anthropological, stable isotope and palaeogenetic analyses. Our aim is to shed light on the relationships of the individuals of the ninefold burial and the circumstances that lead to the death of these people. In order to evaluate biological kinship DNA was extracted from bone and tooth samples of seven individuals. Mitochondrial haplotypes and haplogroups were identified by sequencing of the hypervariable segments I and II of the control region and by analyzing 22 diagnostic coding region single nucleotide polymorphisms. We were able to obtain reproducible endogenous DNA from all individuals investigated. Among these seven individuals we found three different mitochondrial lineages ascertained to distinct haplogroups suggesting maternal kinship among the individuals in the ninefold burial.Combining the results of every discipline of the ongoing project, it is currently not possible to define the circumstances of death. However, several burn marks on the bones of the individuals as well as other signs of violence seem not to be caused by a catastrophe and lend support for a violent raid or a ritual mortuary practice. Further analyses will show, whether the Salzmünde people have been victims of an act of war or ritual practices.

Establishment of mitochondrial SNPs for the investigation of skeletal material buried in the ground

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In forensic and anthropological work on cases of degraded skeletal materials, the analysis of mitochondrial SNPs is of vital importance; this will be shown in a spectacular skeleton find on the premises of the prison in Chemnitz, found in 2002 through excavation works. For each individual, the investigations were carried out at cranial and postcranial parts of skeletons, which had been buried in the ground for about 60 years, and the respective teeth, as far as available. Following the appropriate mechanical cleaning and crushing, DNA isolation was carried out by a combination of the All-tissue DNA kit of the enterprise GENIAL and a DNA cleaning by phenol chloroform. The quantities of bone and tooth manure included in the DNA isolation ranged from 0,8 g and 2,5 g. After inquiry in the literature (Brandstätter et al., Vallone et al.) 24 SNPs were chosen from the coded and non-coded region of the mtDNA. For single base extension, SNaPshot Multiplex Kit (Applied Biosystems) was used. The analysis of the samples was carried out with the ABI PRISM™ 310 Genetic Sequenzer. With this applied DNA separation method it was possible to typify mitochondrial SNPs of skeletal material, which even with STR-analysis is difficult to investigate. This established method is the basis of ancestry investigations with medieval skeleton finds.

Huns in Bavaria? Genetic analyses of an artificially deformed skull from an early medieval cemetery in Burgweinting (Regensburg, Germany)

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The morphological examination of an early medieval burial site in Burgweinting, which is dated to the end of the 5th century, revealed one female with an artificially, circularly deformed skull, a practice that is thought to be associated with the arrival of Nomads of the Eurasian steppe, particularly the Huns.

Individuals with such artificial cranial deformations also can be found in other Late Roman and Early Medieval cemeteries in Europe mostly in the Carpathian basin but only as few isolated cases in Western Europe, where mostly women show such deformations.

Regarding the artificial cranial deformations it is unclear whether a foreign custom was taken over by Germanic tribes or whether the individuals were members or descendants of Eurasian nomads.

With the help of the find of Burgweinting, we exemplarily investigated this question.To identify the possible foreign origin of this female with alleged “Asian” skull deformation we sequenced the HVRI and HVRII region of the mitochondrial DNA.

Our results show that the ancestry of a woman with artificially deformed skull can be linked to an at least partly Asian origin. So this indicates that at least some of the few individuals with skull deformation had not adopted the costume but can be seen as former members or descendants of the hunnish tribal community.

Assessment of the origins of ancient Croatian remains through mitochondrial DNA interpretation

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Croatia as a modern country is relatively new, stemming from the disbanding of Yugoslavia, but the roots of its people span back for thousands of years. In the course of that time varied populations of people have passed through the area, each leaving their mark on the mitochondrial DNA (mtDNA) of the region. While the population of the country is primarily Croat now, there are various other groups such as Serbs residing on the land as well. A look at the mtDNA of past residents will portray the influence of the nomadic Slavic groups of the region, as well as that of previous Germanic and Frankish rule. Mass graves found in the early 1980s have been unearthed as the country led itself into a more modern future, yet the questions of the origins of these remains are still largely unanswered. This joint project between the Pennsylvania State University and the University of Split in Croatia will assess mtDNA profiles from remains of a Croatian gravesite in Šopot-Benkovac. While 10 samples have been analyzed so far, extracted by demineralization and targetting the HV1 and HV2 regions of the mitochondrial genome, inhibition was observed in preliminary testing. This is a common occurrence in ancient bone, along with DNA degradation, so to combat the inhibition we have conducted extractions with the Prepfiler BTA DNA Extraction Kit from Applied Biosystems. Initial results from the use of this kit have indicated a promising reduction in the inhibition, wiwith sufficient DNA yields for analysis. Haplogroups observed thus far are H1 and U. The ancestral route of the Croat people has not been completely elucidated, and so much can still be learned from these uncovered bones and teeth.

Genetic relationship between modern populations and the Neolithic Tyrolean Iceman

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After its discovery in the Italian part of the Ötztal Alps in early 90s, numerous archaeological, biochemical and genetic studies have been concerned with the mummified body of the Tyrolean Iceman, an individual who lived in the south ridge of the Alpine area during the Copper Age (about 5,300 y.a). However, some important questions remain unresolved. The key aspect regards the genetic relationships between the Iceman and modern populations. In fact, recent study on the complete genome of the mitochondrial DNA showed that the Iceman belonged to a branch of haplogroup K1 (named K1f or K1Ö defined by two specific mutations, the 3513T and 8137T), that has not yet been found in extant populations. These results suggests that this lineage could be now extinct or very rare. However, this study was limited by the scarcity of data from modern European populations, especially from the Alpine region of interest. In the framework of the ongoing project “Reconstruction of the peopling of Eastern Alps by analyzing the genetic variability of modern populations and comparison with ancient DNA data” we are analyzing the complete mtdna genome of K lineages (at least 50) from different areas of oriental alps and collecting all complete K mtdna data available from literature. The genetic data will be analyzed in order to get an updated phylogenetic tree of haplogroup K in Europe and to test the presence of lineage related to the Iceman.

(The project is supported by the “Provincia Autonoma di Bolzano – Alto Adige, Ripartizione Diritto allo studio, università e ricerca scientifica, Postdoctoral Research Fellow to V. C).

Molecular genetic analyses of skeleton excavated from Auersperg Chapel archaeological site in Slovenia

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In 2009 the archaeologists excavated five skeletons from the 17th century archaeological site in Ljubljana. They were found in the side chapel of the church in the Franciscans monastery which was the Auersperg tomb. Beside the skeletons the bronze bowl with the heart was found and the name of Ferdinand II and the year of death (1655 - 1706) engraved. The Auersperg (Turjaški) were the most influential aristocrat family on Slovenian territory and one of the richest in Hapsburg empire. In 2011 we have been asked for identification of five skeletons excavated from the Auersperg chapel. Skeletons were badly remained and bones degraded to small peaces. Fragments of femurs and teeth were preserved only for two skeletons and for the rest of three skeletons the fragments of cranium were used for molecular genetic analyses. We cleaned the bones and teeth, removed surface contamination, and ground them into powder using liquid nitrogen. Prior to DNA isolation bone or tooth powder was decalcified. The nuclear DNA of the samples was quantified using real-time polymerase chain reaction. We extracted up to 10,7 ng DNA/g of bone and tooth powder from Auersperg chapel archaeological site skeletal remains. We obtained complete genetic profile of autosomal DNA, Y-STR haplotype, and mtDNA haplotype for HVI and HVII region from one skeleton. For traceability in the event of contamination, we created an elimination database including genetic profiles of the nuclear and mtDNA of all persons that had been in contact with the skeletal remains and no match was found. We are waiting for the family reference samples for comparison with genetic profiles obtained and for identification of the skeleton excavated from Auersperg chapel archaeological site. This is the first archaeogenetic research in Slovenia.

DNA analysis of lineage markers (mtDNA and Y-chromosomal STRs) on ancient or aged bone samples

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Bone samples belong to the group of the most challenging samples we can face during our forensic practise. Criminal cases involving up to 20-years old skeletons are not rare and having a robust and reliable DNA extraction and STR typing method is a must. Work with the ostheological specimen that is several hundred years old is even more challenging and the chance of false negative or false positive identification results is increasing with the longer post-mortem interval and bad storage conditions. The authors of this talk will demonstrate how can be the DNA analysis of lineage markers (mtDNA and Y-chromosomal STRs) utilized during the process of DNA based identification of ancient or aged bone samples.

Forensic genetic analysis for the AmpFlSTR Yfiler system in the Korean population

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Y chromosome short tandem repeats (STRs) are a powerful tool for forensic purposes and evolutionary studies. We have analyzed variation of 17 Y-STR loci contained in the AmpFlSTR Yfiler PCR amplification kit in a sample of 105 unrelated individuals for the same samples recently typed for a set of rapidly-mutating Y-STRs (RM Y-STRs) from Korea. Allele frequencies and forensic parameters have been used to evaluate suitability and robustness of the kit for forensic genetic analyses. A total of 103 haplotypes were identified, 101 of which were unique. Total haplotype diversity was greater than 99.96% for the Korean population. The lowest gene diversity studied was 0.333 for DYS391 and the highest 0.968 for DYS385a/b. Discrimination capacity was 98.10%. Multidimensional scaling (MDS) plot of genetic distances (ΦST) calculated from allele frequencies of the 17 STR loci with published data showed that the Koreans appeared to have the most genetic affinity with the Vietnamese, followed by Japanese and Mongolian Khalkh of the East Asians but tend to be different from the Europeans. Our data, therefore, can be used to extend the results obtained with other STRs, as well as provide valuable information for forensic and population genetic studies in the Korean population.

Genetic polymorphisms of 19 STR Loci in the Chaoshan Han population

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[**Objective**] To investigate the genetic polymorphism of the 19 short tandem repeat(STR) loci D8S1179, D21S11, CSF1PO, D3S1358, D7S820, TH01, D13S317, D2S1338, D18S51, D16S539, TPOX, vWA, D19S433, D5S818, FGA, PentaD, PendaE, D12S391, D6S1043 in Chaoshan Han population, and to compare the forensic application of the three different kits: PowerPlex 16□Identifiler and Sinofiler. **[Methods]** Allele frequencies for 19 STR loci were determined in a sample of 1004 unrelated Chinese individuals from Chaoshan area of Guangdong Province, a littoral located in the southeast of the Mainland China. The allele frequencies and statistical analysis were performed using the Modified-powerstate program. **[Results]** A total of 227 alleles and 872 genotypes were detected. The allele frequencies vary between 0.001 and 0.570, the genotype frequencies between 0.001 and 0.347, the observed heterozygosity (Ho) between 0.588 and 0.884, and the polymorphic information content (PIC) between 0.51 and 0.90. No deviations of the observed allele frequency from Hardy-Weinberg equilibrium expection were found for Chi-square test (P>0.05) except D2S1338 loci. Total discrimination power (TDP) of 19 STR loci is 0.99999999999999999999316072. The TDP of PowerPlex 16, Identifiler, and Sinofiler are 0.99999999999999999454124,0.99999999999999999887466,0.99999999999999999952528, respectively. The cumulative probability of paternity exclusion (CPE) for triplet cases with 19 STR loci is 0.9999999868343, and the CPE of PowerPlex 16, Identifiler, and Sinofiler are 0.99999870529, 0.99999823985, 0.999999682455, respectively. **[Conclusion]** All 19 STR loci showed highly polymorphic in Chaoshan Han population. The three kits are all suitable for forensic work for the Chaoshan Han population, among which Sinofiler is the most powerful system. The allele frequencies reported in this study would serve as a reference database for personal identification and paternity testing.

Central Croatian population data of eight X-linked markers in four linkage groups

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The analysis of short tandem repeats (STRs) located on X chromosome shown to be particularly powerful in solving complex kinship cases. The aim of this study was to analyze 8 X-STRs in central Croatian population. We carried out a statistical analysis of the data from previously performed genetic analyses collected during routine forensic work by the Forensic Science Centre ''Ivan Vučetić''. A total of 99 unrelated healthy women and 78 men from central Croatia were typed using Mentype Argus X-8 PCR amplification kit. The allele and haplotype frequencies were determined by counting. Haplotype frequencies were calculated only in male samples. Arlequin 3.5 software was used to assess Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD), observed and expected heterozygosity. For HWE and LD tests Bonferroni correction was used. Power of discrimination (PD) for males and females was calculated according to Desmarais. Polymorphism information content (PIC) was determined using online database ChrX-STR.org that calculates population-genetic data. In female samples deviations from HWE ($p>0.00625$) for each locus were not found. LD test performed on female and male samples, revealed no significant association between markers ($p>0.00178$). In 78 men, 37, 30, 35 and 30 haplotypes were found for linkage groups 1-4. Locus DXS10135 was the most polymorphic (PIC=0.9306). DXS7423 and DXS8378 loci showed the lowest values (0.6316 and 0.6447). PIC for whole marker set was 0.999998. PD varied from 0.6922 to 0.9345 in male and from 0.8447 to 0.9918 in female samples. Combined PD reached 99.9999% in males and 99.99999999% in females. Further analyses that will include more X-STR loci are needed to increase discrimination power for kinship and paternity testing as well as population genetics studies. Nevertheless, Mentype Argus X-8 kit can be used as an additional marker panel for forensic identification and complex kinship cases in central Croatian population.

Canine mitochondrial genome sequencing to improve the genetic profiling of dog hair

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Genetic identification of traces of non-human origin is becoming increasingly important in forensic casework. Dog hairs are frequently discovered on crime scenes and on the clothing of victims or suspects. Given that nuclear DNA analysis is impracticable for hair shafts, the profiling has to focus on mitochondrial DNA (mtDNA).

Previously, the control region of the canine mitochondrial genome (mtGenome) was explored at the NICC. A Belgian population database was assembled and showed sufficient diversity for application of control region analysis of dog hairs in forensic casework. Nonetheless, its discriminative power is smaller compared to the human control region. Moreover, the frequent occurrence of a number of haplotypes in the population is disadvantageous for the evidential value of the analysis, an observation made in several other studies worldwide.

The objective of the current project is to improve the discriminative power of the mtDNA profiling of canine trace material by exploiting the genetic information in the coding region of the mitochondrial genome. A method was established to sequence the entire canine mtGenome according to QA standards, based on only 2 overlapping amplicons of about 9 kb each and double strand sequence coverage that determines each position at least twice independently.

The mtGenome sequences of about 125 dogs selected from the previous population study were assembled. These sequences improve the resolution of the phylogeny of the canine population. Applying a phylogenetic approach, the informative positions of the mtGenome that contribute most to the improvement of the discriminative power of canine mtDNA analysis can be determined.

The whole mtGenome structure should enable us to develop an improved sequencing strategy for mtDNA profiling of dog hair. Since whole mtGenome sequencing is unfeasible for trace material, alternative approaches will need to be used such as SNP-multiplexes that focus only on identifying a number of informative sites.

Is the Indian Wild Boar an evolutionary significant unit? Molecular insight into phylogeny of the Wild Boars and Domestic Pig

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We examined genetic variations among wild pigs from India and compared their molecular phylogeny with those of domestic pigs and wild pigs of non-Indian races by analysing the sequence of the mitochondrial cytochrome-b gene. Our study revealed unambiguous genetic variations between the Indian wild pig and the domestic pig. Our study also differentiates the Indian Wild pigs from the wild pig races of the world. It is evident that the Indian wild boar is a unique species or at least an incipient species that exhibits a high degree (more than 3%) of genetic variation within the evolutionarily conserved cytochrome-b gene compared with other wild boar races. The phylogenetic tree indicates that domestic pigs in India are not originated from Indian wild pigs. We conclude that the 3% nucleotide difference between the Indian wild pigs and domestic pig is helpful in differentiating between them as well as differentiating them from other wild pig races. This strategy is helpful in identification of the confiscated biological sample of these two subspecies for unambiguous differentiation of the wild and domestic pigs for solving the wildlife forensic cases.

Investigator Plus – fast, sensitive and robust amplification of common standard set loci

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Forensic DNA laboratories are challenged by the requirement to provide results on the identity of genetic evidence within a very short time. Thus, in addition to crucial quality parameters like sensitivity and robustness, speed becomes an increasingly important feature of STR PCR assays. We have developed a set of next generation Investigator Plus kits that combine all critical features necessary for fast and reliable analysis of demanding forensic samples: ESSplex (European Standard Set), ESSplex SE (including SE33), and IDplex (CODIS). Based on our fast-cycling PCR technology, we have introduced a novel reaction mix that allows completing a standard 30 cycle amplification in as little as 90 minutes. Using this protocol, well balanced full profiles can reliably be obtained with 100pg of DNA template. All Plus assays are very robust towards potential PCR inhibitors and can tolerate concentrations up to 200ng/µl humic acid, or up to 750µM hematin. They provide a clean baseline without any dye artifacts. We furthermore will show results on direct amplification from FTA paper and buccal swabs. The combination of all features mentioned above helps to reduce the number of samples that have to undergo reanalysis, which further contributes to more streamlined and efficient laboratory workflows.

How to improve STR analysis using a novel quantification technology: more than DNA quantification

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Quantification in forensic casework analysis is typically the only pre-STR analysis step and could be seen as a quality control step rather than just a quantification of the DNA concentration.

Thus, we present data from our novel human DNA quantification assay product line, called Investigator® Quantiplex Kits, that is available as human (Quantiplex Kit) and human/male (Quantiplex HYres Kit) configuration. Both provide fast and accurate quantification of DNA in forensic database and casework samples with a very high sensitivity and accuracy due to the trusted and validated autosomal multi-copy target 4NS1C and the validated multi-copy target on the Y-chromosome. Detection of inhibitors is ensured by a balanced internal amplification control, without significantly affecting the DNA quantification results. This novel technique of high quality control standards provides a highly accurate assay for DNA quantification in forensics. The assay ensures an improved correlation to the STR results in comparison to other standard real-time PCR methods.

The Investigator Quantiplex product line utilizes a novel PCR fast-cycling technology as well as Scorpion primers that enable rapid results. Using the Rotor-Gene Q system, quantification can be performed in around 50 minutes.

Improving DNA sensitivity and strengthen reliability with low-level DNA testing using trace amount of metal

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The amount of DNA recovered from a crime scene may often not be enough to obtain a full profile using conventional multiplex kit. Improved sensitivity in a detection technique is a usually a valuable cue to enable results to be obtained from limited biological evidence. Without the increasing the ability to make copies of DNA, many forensic samples would be impossible to analyze. However, DNA from crime scenes is often limited in both quantity and quality and including contamination. So, we presented new efforts for improving low-level DNA testing. Materials and method We used commercially available 9948 male control DNA (Promega) as the PCR template (50pg). In addition, trace amount of various biological samples were tested. Amplification were done by the addition of various trace elements with the AmpFLSTR™ Y filer and PowerPlex™ Y System according to the manufacturer's instructions,using the AmpliTaq Gold. In order to evaluate the assay sensitivity, appropriate concentrations of metal (As, Cd, Cu,Hg, Pb, Mn, Tl, Ga, Se, V, Zn, etc.) were added to the buffer in a dilution series from 0.01-1.0 µg/ml. The effect of metal to enhance the PCR reaction was quantified according to the manufacturer's protocol on an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems). Results We found that some metals (Cu, As, Zn etc.)remarkably enhance PCR. The enhancement effect is found at a range of concentrations, from 0.01 µg/ml to 1.0 µg/ml in the reaction mixture. The most effective concentration is0.2µg/ml (200ppb) in reaction mixture. We will present the detailed data in the meeting.

Sample lysis and DNA separation in single tube assemblies for accurate forensic profiling

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The importance to improve the quantity and quality of DNA isolated from forensic samples is without controversy. Extraction of genomic DNA forms the first step of DNA profiling and its quality is most critical for all subsequent steps to increase the potential to obtain maximum information from downstream Short Tandem Repeat (STR) analysis. It starts with the disruption of the cellular structure to create a lysate and the separation of the soluble DNA from cell debris and other insoluble material to prepare a DNA lysate for further purification. Overall efficiency will be improved, and the yield of isolated DNA will be optimised by carrying out the lysis directly in a single tube assembly with subsequent quantitative recovery of the lysis buffer from both routine and challenging samples.A novel kind of extraction systems was developed that allows simple and fast separation of substrate from lysate in an all-in-one system. This approach eliminates the manual lysate and substrate transfer steps, saving time and minimizing cross contamination and sample transposition events significantly. Flexibility of the systems in terms of lysis conditions and required reagents was demonstrated using a variety of lysis protocols. Small evaporation rates in vapor tightness tests confirmed that these systems are ideally suited for incubations at higher temperatures and over longer periods too. The SQ version rationalizes the lysis portion of DNA extraction method and overcomes obvious difficulties with commonly used methods. The performed study proved their suitability for effective DNA preparations even from low sample inputs providing particle-free DNA lysates of highest quality and maximized yield. Significant time savings and improved reproducibility were demonstrated too. Gradual DNA extraction in a single tube assembly represents the unique characteristic of the DL version for differential lysis of mixed specimens. Differential lysis was shown under mild conditions to separate the female DNA and harsher lysis conditions that break the spermatozoa within the same filter column and without necessity of sample carriage. The DNA lysates were further purified and used to generate autosomal STR profiles of both the victim and the perpetrator. Due to high yield of DNA, the chance of a successful DNA-profile by downstream analysis was significantly increased. Thereby, simple handling allows timesavings and higher throughput in a manual process to allow reliable improvement of crimesolution rates and showed its qualification to speed up analysis of backlogged crime samples.In summary, universal DNA extraction systems were developed to provide particle-free DNA lysates of highest quality using a diversity of specimens including forensic sample material. Improvements of overall efficiency, in particular maximizing the performance of the early steps of the extraction method was shown to achieve better genotyping results.Furthermore, the DL version demonstrated the potential as an improved methodology toovercome the often claimed difficulties in differential extraction, thus, has the potential towork off backlogs of rape kits.

Estimating forensic match probabilities for Y-STRs using a new, discrete Laplace distribution

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The evidential weight of Y-STR investigations in crime cases may be expressed as the likelihood ratio Pr(Y-STR-profile | H_0)/Pr(Y-STR-profile | H_1), where Y-STR-profile is the profile of the stain found at the scene of crime and Pr(Y-STR-profile | H_1) is the probability of the Y-STR-profile among random individuals. Even with a limited number of STR loci investigated, the majority of Y-STR-profiles in small database samples are only found once. This indicates that, if the Y-STR-profile has not been observed before, a naïve estimate of the probability of the Y-STR-profile, like 1/n or 1/(n+1), where n is the total number of individuals in the database sample, most likely is overestimated. We present a new method that is inspired by Fisher-Wright theory and an assumption of discrete Laplace, 'wandering' distribution of the Y-STR-profiles.

A simulation study of populations created according to the Fisher-Wright model with fixed expected end population sizes, two initial population sizes, two mutation rates, two different numbers of generations was performed. This was repeated five times in total for each combination. Fifty databases with 500, 1,000 and 5,000 Y-STR-profiles, respectively, were randomly sampled from each population. The total number of data sets was 6,000.

In the simulated data, 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 estimate method and Brenner's kappa method.

The simulation method is implemented in R and public available on http://cran.r-project.org/web/packages/fwsim/index.html and the discree Laplace estimation method on http://cran.r-project.org/web/packages/disclapmix/index.html.

Thus, under the assumption that the Y-STR-profiles are distributed according to the Fisher-Wright model, the discrete Laplace estimation method for estimation of Y-STR-allele probabilities seems to give the best estimates when compared to other suggested estimation methods.

Evaluation of the use of software as a tool in checking mitochondrial DNA HVI and HVII sequences analysis

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The work presented here discusses the use of the Mitotyper ™ software (presented by Elling & Den Hartog in 24th World congress of ISFG 2011) to check the sequences analyses in order to avoid potential problems, such as errors describing the sequence difference-from –reference polymorphisms.

We analyzed the HVI and HVII regions in a sample of 124 not related individuals from São Paulo, Brazil. The first analyses were performed by two independent analysts as previously described Godoy et al (Forensic Science International Genetics Supplement Series e149-150, 2011). We re-analyzed these samples with the Mitotyper ™ software reviewing and comparing the polymorphisms found. The program detected polymorphisms in four samples that had not been observed in the previous two manual analyses. In one additional sample, a more accurate description of the polymorphism, in the software analysis, allowed the haplogroup to be more specifically classified. The divergent manual results found**- 5 of 124 (4%)-** were due to the interpretation and experience of the third analyst in manipulating the raw electropherogram data.

We conclude that Mitotyper has all the rules for the sequence analyses and is a good tool to help to check and validate the results. The software is useful for efficiently checking the sample analysis in routine research and casework, allowing the users to double check the analyses, saving time for the manual and laborious analysis in divergent samples. However, good quality eletropherograms are still essential for correct sequence analysis. (**FAPESP 2010/19127-2, CNPq, Capes**)

The effect of sample size on the estimates of mtDNA genetic diversity parameters in isolated European populations

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The study of human genetic Isolates provides an opportunity to analyze the effects of geographical and cultural factors on the genetic structure of human populations. Numerous studies have investigated genetic Isolation in Europe, focusing on fluctuations of measures of genetic diversity . Unfortunately, some of the features often associated to isolation (small census sizes and high degree of endogamy) may also limit the number of unrelated individuals which is possible to collect. Thus, it becomes difficult to disentangle the effect of genetic isolation from those created by inadequate sample size. In the present study we evaluate how sample size may affect estimates of diversity measures through a reanalysis of the current mitochondrial data on European genetic isolates. We retrieved genetic data of 24 populations characterized by geographic and/or cultural isolation and compared them with open European populations for several intra and inter population diversity parameters (HD, MNPD, Fst, Fu’s Fs) using a random resampling approach. Our results shows that these measures may differ in robustness and informativity. Reviewing published studies in the light of our approach, we suggest that care should be taken when drawing inferences of genetic isolation using small sample sizes.

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Genes, mountains and culture: evidence for the impact of ethnicity on the structure of human populations

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In the current anthropological approaches, ethnicity is a symbolic construction produced by specific historical, social and political circumstances. Individuals belonging to a given ethnic group tend to self-attribute a reciprocal similarity, both biological and cultural, and a diversity compared to neighbors. In the long term, this may lead to a reduction of intra-group genetic variability associated with endogamy and genetic drift. In this study we assess the impact of ethnic boundaries on genetic structures in European populations, using mitochondrial control region data. First, we investigated the degree of genetic differentiation between three german-speaking neighboring populations of the north-eastern Italian Alps: Sappada, Sauris and Timau. These populations share many cultural aspects, but their components do not self-identify as belonging to the same community. Thereafter, we compared the results obtained with those of other well defined ethnic groups, Cimbrians and Ladins from north Italy and Aromuns from Albania and Macedonia. We observed in all groups a reduced genetic diversity and a high differentiation with respect to other neighbouring and European populations. These analyses highlighted a higher diversity among the german-speaking populations of the north-eastern Italian Alps than Cimbrians, Ladins and Aromuns, pointing to the importance of ethnicity as a factor shaping genetic structure in human populations.

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Critique of the haplotype surveying method

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A method called “haplotype surveying” has been proposed to assess the rarity of a Y-haplotype and is often taken to be a valid calculation for forensic evidence connecting suspect to crime scene. I am skeptical of the method for several reasons.

One – Wrong statement of the problem, and no explicit model.

Haplotype surveying is presented as a method to estimate frequencies, but that is not the same as the normally relevant evidential question of a conditional (upon having observed the crime scene type) match probability of an innocent suspect. Careless definitions can have real and detrimental consequences. For example, the first published version of haplotype surveying forgot to consider the crime scene type.

The “survey” idea that the popularity of a haplotype is related to that of its neighbors hints at a model involving mutation, but nothing is explicit and there is no indication of the nature of the process relating incidences of neighboring types. Consequently it is perhaps not surprising that, as Veldman noted

Two – The implied model not correctly implemented.

The weighting formula presented in the model treats a distance of two mutations between a pair of haplotypes almost the same as a distance of three and not much differently than a distance of one. I do not see what model would give rise to the formula.

Three – Drift, not considered, likely overwhelms the neighbor correlation concept.

An explicit statement of the intended model would be helpful, but the method seems to assume some kind of mutational equilibrium as might exist in an infinite population. Possibly neighboring haplotypes are envisioned as replenishing one another over generations via mutation. But computer simulations suggest that such an effect would be very minor compared to random drift.

Evaluation of haplogroup predicting softwares

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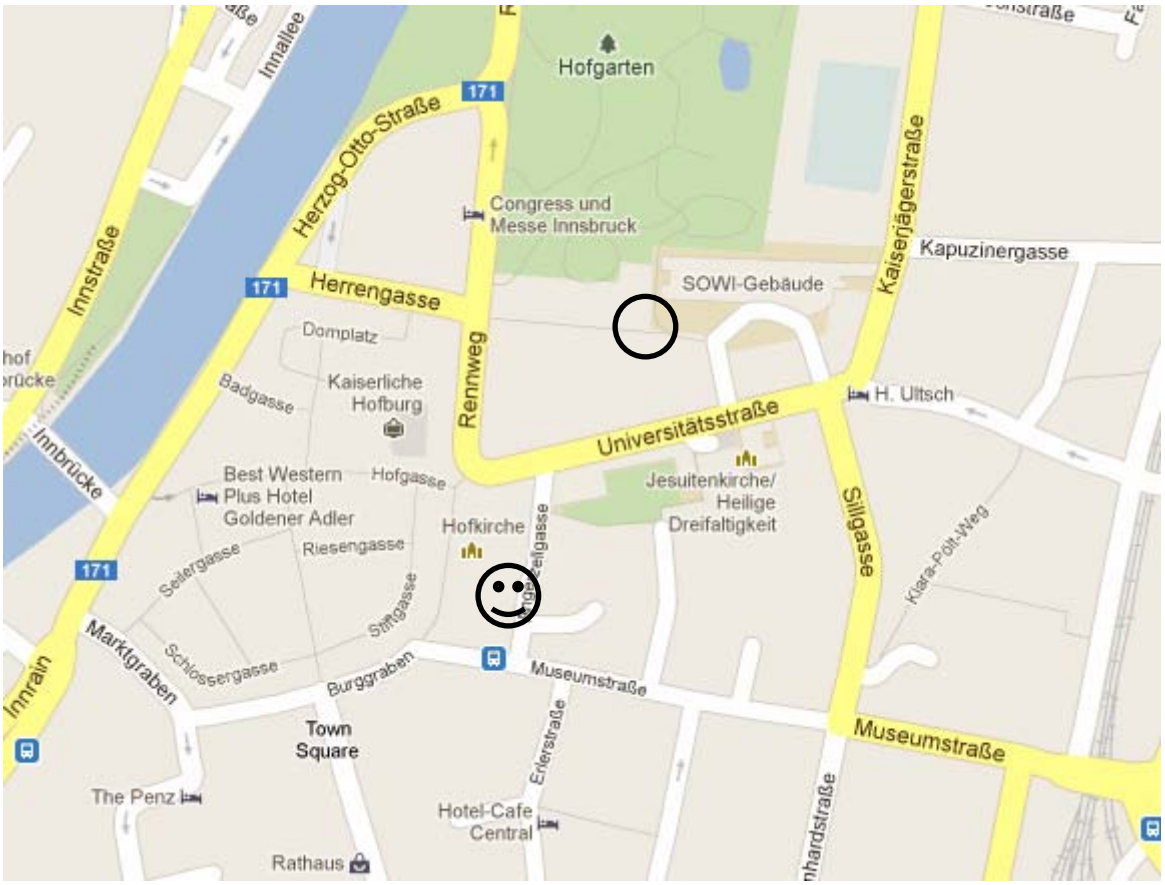
In population genetics studies, huge collections of Y-STR haplotypes have been published; unfortunately most of them lack information concerning the haplogroups to which the haplotype belong. Inferring haplogroups from haplotype data is an attractive alternative, however a robust informatic approach should be used. Based on the most theoretical criticism rised by some authors, we decided to empirically test the Athey's Haplogroup Predictor with samples previously typed for haplogroup defining SNPs. One hundred and one unrelated male samples were haplotyped by AmpFLSTR Y-Filer (Applied Biosystem) and haplogrouped by SNP detection performed by different approaches, namely Real Time PCR followed by High Resolution Melting, amplicon sequencing, SnapShot mini-sequencing and/or primer specific PCR. The analyzed SNPs were M3 for Q1a3a, M269 for R1b1b2 and U179 for I Haplogroups. Thirty one samples were assigned as "not determined" and 70 samples were haplogrouped as I, Q1a3a or R1b1b2. The prediction of Y-Chromosome haplogroups from these samples was performed by Athey's Haplogroup Predictor considering "equal priors". All the haplogroups predicted by the online program matched the assignment by SNP genotyping. An average probability of 99.7%, 96.9% and 99.99% was obtained for Q, I, R1b haplogroup assignment, respectively, using Y-Filer STR panel. An adequate correlation between the haplogroups predicted by the software and SNP haplogroup typing was obtained. The use of Athey's Haplogroup Predictor by means of 17 markers (Y-Filer Panel) showed a reliable accuracy to predict at least I, Q1a3a and R1b haplogroups. Its use might offer an opportunity for retrieving valuable information from published haplotypes. Increasing the number of reference samples from which haplogroup have been precisely defined by SNP analysis will highly improve the software accuracy. Meanwhile, this approach represents an acceptable screening criteria that may allow analyzing previously published results.



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