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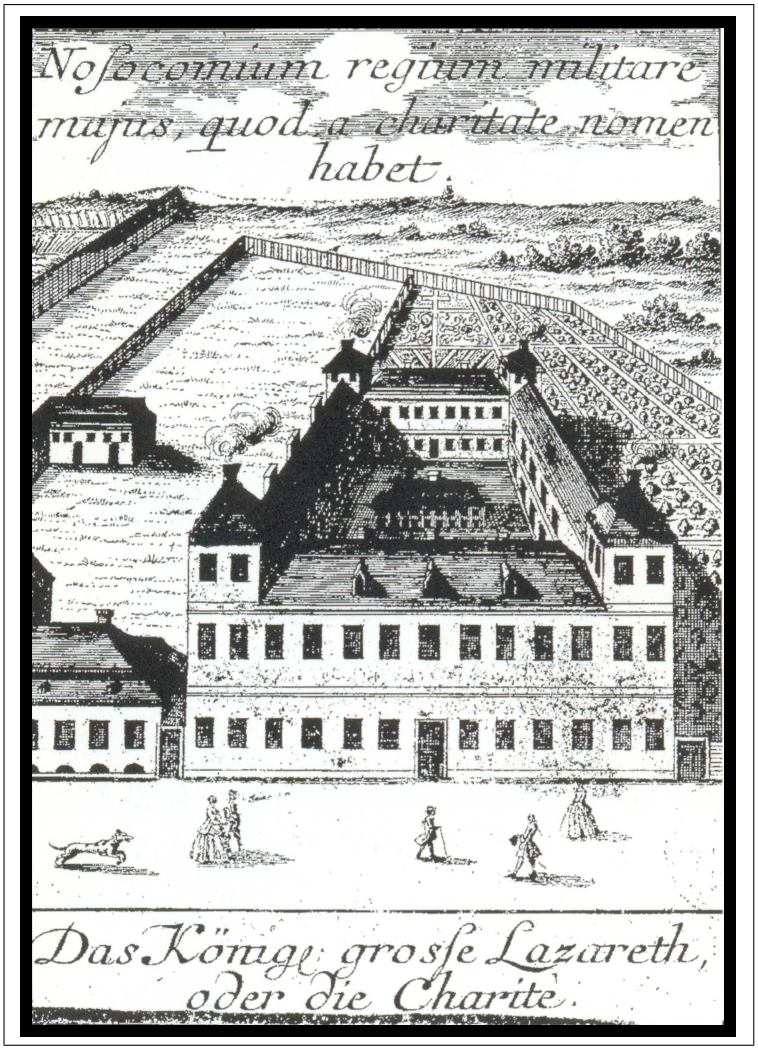

ABSTRACTS
Talks and Posters

IV. International Forensic Y–User Workshop
— Haploid DNA markers in forensic genetics —

Berlin, November 18 - 20, 2004

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Genotyping Y-SNPs: development of PCR multiplexes for forensic applications of the phylogeographic analysis of the Y-chromosome

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The aim of this study was to set up PCR-multiplexes of NRY-SNPs suitable for phylogeographic analysis of Y-chromosome and for the related forensic applications. Taking into account the hierarchical structure of Y-SNPs, they were analyzed in two steps, starting from the most basal branches of the phylogenetic tree to assign evidence for a major clade, and proceeding along to the relevant branches to the shallowest markers to identify the haplogroups inside every major clade. The first step was investigated by 15 basal markers, arranged in a hepta-plex and a octa-plex. Four further multiplexes (2 hexa- and 2 penta-plexes) exploring the shallower branches of the phylogenetic tree characterizing the European haplogroups J2, E3b, R1 and I were also developed. SNP genotyping was carried out by minisequencing using the SnaPshot multiplex technology and extension product typing was performed by capillary electrophoresis. In view of the forensic application of the Y-SNP multiplexes developed in this study, PCR was performed starting from minimal amount of DNA and primers were selected yielding PCR amplicons in the range between 63 and 210 bp. The multiplexes were employed for haplotyping one sample of Italians and experiments on sonicated DNA fragments were also performed.

A study of Y Chromosome microsatellite variation in Gabon

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One of the major cultural expansions in Africa, the Bantu expansion of languages, took place around 5000 years ago and was originated in southern Nigeria and/or northwestern Cameroon. Although the linguistic side has been widely analysed, little is known about the demographic processes associated to this cultural change. The aim of this analysis is to determine the demic apportionment to the Bantu expansion and establish a theory of the origin and diffusion of Bantu and Bantu-speaking populations.
On the genetic side, much is known on sub-Saharan Africa in terms of mitochondrial DNA (mtDNA). However, less is known on its paternal counterpart the Y chromosome, which is also uniparentally inherited. Since the phylogeny of the Y chromosome has been exhaustively described, it is possible to reconstruct a phylogeography of the human male lineages in sub- Saharan Africa.
We have chosen the Gabon area, located in the Guinea Gulf, in order to describe the Y-chromosome genetic variation of the populations located in the area where the western route of the Bantu expansion took place. To achieve this, we have typed 18 microsatellite markers (DYS 389 I, DYS 389 II, gata A7.1, gata A7.2, DYS 385, DYS 462, DYS 19, DYS 388, DYS 390, DYS 391, DYS 392, DYS 393, DYS 434, DYS 435, DYS 436, DYS 437, DYS 438, and DYS 439) in more than 450 samples from different areas of Gabon. This will enable us to identify possible migration routes and study correlations between languages, cultures and genes.
We are also interested in carrying out further analyses using Y-Chromosome Single Nucleotide Polymorphisms providing a further refinement to our results.

Relevance of mtDNA sequence data for the victim identification after the WTC terrorist attack

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Mitochondrial DNA (mtDNA) testing was part of the identification effort from the onset of the project. Instead of selecting only maternal references and degraded samples (remains), a decision was made to perform high throughput mtDNA sequencing parallel to STR typing on all specimens, references and remains. The testing was outsourced to the private laboratory Celera (Rockville, MD).
Benefits from this approach were as follows. Some identifications were found through investigative leads developed by mtDNA matches despite full STR profiles for the remains. An increased number of possible body part associations were made for samples with partial STR types and full mtDNA results. Duplicate independent testing provided Quality Control for sample association and identification, detecting problems associated with STR testing.
The main disadvantages of the bulk testing approach were the additional expense and time required for the interpretation of the high volume of data generated. In many instances mtDNA data were not required to achieve the identification, but the mtDNA results were still reviewed by personnel to assure no conflicts occurred with STR results.
Aspects of testing and specific case examples pertaining to benefits and disadvantages will be discussed.

Phylogenetic tools for quality assessment of mtDNA population data

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Recent studies on the quality of human mtDNA control region databases have shown that a considerable number of published databases contain erroneous profiles. Besides others, clerical errors and crossovers of non-contiguous stretches of DNA, also referred to as “artificial recombination”, were identified as the main source of errors in mtDNA databases. The latter cannot be identified by means of the raw data but require an alternative approach for detection. Here, we present two computer programs that we developed to assign mtDNA control region profiles to established haplogroups and thus be able to detect artificial recombination of mitochondrial DNA fragments deriving from different samples (haplogroups).

Major Y-chromosome haplogroup typing by use of a 29-multiplex kit

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The SNPforID consortium

In the context of the European Consortium "SNPforID High throughput analysis of single nucleotide polymorphisms for the forensic identification of persons", a panel of 29 Y-chromosome SNPs has been selected for the identification of the major population haplogroups. With this panel of SNPs it is possible to discriminate between the major Y chromosome haplogroups present in the world. However, only some of these groups will be present in European populations. To provide discrimination between European populations, an additional selection of markers will be required. Depending on the exact composition of the populations normally encountered by any one forensic lab, different multiplexes may be required in combination to assign a sample to a specific haplogroup and more accurately predict geographic origin.
All 29 SNPs were amplified in a single PCR reaction and analysed in a single minisequencing reaction using the SNaPshot typing kit (Applied Biosystems). A validation was performed, firstly to check the accuracy and reproducibility of the 29-plex in different laboratories, and secondly to obtain haplogroup frequencies in samples from the major population groups.
To compile the sample collections each of the participating groups reported the samples they had available in their labs. Among all the populations reported, a set of 1670 male samples distributed in 17 populations was selected. This selection was performed to obtain the best possible representation of the general worldwide distribution of populations. Selected population samples were distributed equally among the participating laboratories to perform the validation as a collaborative exercise. The data will be compiled in a central database, available at the consortium webpage (`www.SNPforID.org`) as soon as possible.

Y-Chromosome and Mitochondrial DNA Work at the U.S. National Institute of Standards and Technology

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At the U.S. National Institute of Standards and Technology (NIST), we have developed a Standard Reference Material for human Y-chromosome analysis, SRM 2395, which enables calibration of Y-STR results and nomenclature across laboratories worldwide [1]. SRM 2395 includes 5 male DNA samples selected to exhibit a diverse set of alleles across the commonly used Y- chromosome short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers. A female DNA sample is also included to serve as a negative control for male-specific DNA tests. The five male samples in SRM 2395 have been sequenced at more than 20 commonly used Y-STR markers (in both forward and reverse directions) to confirm allele calls. The Y-STR loci typed and sequenced for SRM 2395 include DYS19, DYS385a/b, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS435, DYS436, DYS437, DYS438, DYS439, DYS447, DYS448, DYS460, DYS461, DYS464 a/b/c/d, Y-GATA-H4, DYS635 (Y-GATA-C4) and YCAII a/b.
A set of approximately 650 population samples from U.S. Caucasians, African Americans, and Hispanics have been characterized across the above listed loci [2]. Examination of additional Y-STR loci with these same samples is being performed and analysis of different haplotype combinations will be presented illustrating optimal sets of markers to resolve samples matching at the minimal haplotype and U.S. haplotype loci. In addition, concordance studies have been conducted on these samples with in-house multiplex assays and commercial Y-STR kits. A subset of these samples has been characterized with 50 Y-SNP loci covering the 18 major Y-chromosome haplogroups [3]. The impact of Y-chromosome gene duplication on assay design [4] and mixture interpretation will also be reviewed. Information on commonly used Y- chromosome markers has been standardized and made available over the Internet through the NIST STRBase website: `http://www.cstl.nist.gov/biotech/strbase`.
In collaboration with the Armed Forces DNA Identification Laboratory, multiplex assays examining forensically useful mitochondrial DNA (mtDNA) coding region polymorphisms have been developed [5]. Results from Roche Molecular Systems' mtDNA HV1/HV2 LINEAR ARRAY screening assay across our U.S. population sample set will also be described [6]. NIST provides a Standard Reference Material for human mtDNA sequence information, SRM 2392- I (HL-60 cell line DNA), which has been characterized across the entire mitochondrial genome [7].

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Development of a Male-Specific, 12-Locus Fluorescent Multiplex

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Short Tandem Repeat (STR) analysis has become the leading technology for genetic human identification. Frequently, autosomal markers are used for forensic, paternity and anthropological studies. However, some cases can benefit from the analysis of sex-specific Y-STR markers. Y-STR markers consist of polymorphic regions found on the non-recombining region of the Y chromosome. Amplification of these haploid markers occurs only in males and alleles are inherited only through the paternal line. These qualities simplify interpretation of complex male/female mixtures and male kinship studies by removing the female contribution.

Several web-based databases of observed Y-STR haplotypes have been initiated (`http://www.yhrd. org/`). These databases include the so-called "Y-STR minimal haplotype", which consists of nine loci: DYS19, DYS385I/II, DYS389I/II, DYS390, DYS391, DYS392, DYS393. A commercially available, single-amplification assay for these loci has yet to be offered. To this end, a fluorescent multiplex has been developed to include the Y-STR minimal haplotype plus DYS437, DYS438 and DYS439. This new PowerPlex® System uses four-color chemistry allowing analysis on the ABI PRISM® 377 DNA Sequencer, ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 3100 Genetic Analyzer. Amplified samples are labeled with fluorescein, 6-carboxy-4',5'- dichloro-2',7'-dimethoxy-fluorescein (JOE) and carboxy-tetramethylrhodamine (TMR). Fragment sizing is provided by an internal size standard labeled with carboxy-X-rhodamine (CXR). Color deconvolution can be performed with color matrix kits currently available from Promega Corporation. Allelic ladders have been created, following ISFG recommendations, to increase confidence in allele designation. A PowerTyper macro, operating within the Genotyper® software, has been designed to automatically label fragments from GeneScan® data using the supplied allelic ladder and size standard. Primers have been designed to yield amplification products that are less then 350 bp in length. System sensitivity, specificity, robustness and concordance with previously described primer sets will be discussed.

Distribution of Y-chromosomal Haplotypes for the Three Major Ethnic Groups (Malays, Chinese and Indians) in Malaysia

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A combination of eleven Y-linked microsatellite loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS438, DYS439) and Amelogenin, were amplified simultaneously using the Reliagene Y-Plex™12 (New Orleans, LA) in 834 Y-chromosomes from three major ethnic groups within the Malaysian population. We typed 277 Malays, 266 Chinese and 291 Indians on the standard ABI™ 3100 Genetic Analyzer for genetic variations in their Y-chromosome lineages. The distribution and haplotype frequencies of the Y-specific loci were determined and compared.

A total of 738 haplotypes were found in the three ethnic groups, out of which 671 were unique, i.e. 91% of the observed haplotypes only occur once in the three groups. Haplotype diversity was highest in the Malays (0.99996) followed by the Indians (0.9989) and the Chinese (0.9985). Locus diversity values ranged from 0.332 to 0.866, with the highest values observed in DYS385a/b (> 0.7) and the least in DYS391 (< 0.50). Significant differences in the distribution of the haplotypes between the three groups will be presented. A notable proportion of Y-STR rare alleles was detected in these three Asian ethnic groups. Additionally, the population study also revealed a higher frequency (2.1%) of a rare null Y-allele at the Amelogenin gender locus in the Malaysia Indian group compared to the other two ethnic groups (0.4% and 0%).

This Y-STR Haplotype Database is specific for the Malaysian population and will be used in forensic casework by the DNA/Serology Laboratory of the Forensic Division in the Department of Chemistry, Malaysia.

Evaluation of the Ethnic Composition in the Population of Argentine

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In the early XVI century, at the moment of the Spanish conquest, the territory of Argentina was inhabited by an Amerindian population with an extremely low population density. Accordingly it was supported by anthropologists that the Aboriginal component was in the past and is at present extremely scarce, considering that most inhabitants are of European ancestry and hence, Argentina and Uruguay claim to be the "European countries" of Latin America. In addition, it is accepted that the overall Amerindian population at the moment of the conquest was about 400,000 people. Nowadays, it is recognized by the government authorities that the Aboriginal component is less than 200,000 people, representing less than 0.5% of Argentina's population. Demographic estimations, census and counting may provide biased information. Some molecular analysis carried out on blood groups denoted quite different results compared with the anthropological data. In order to investigate the actual ethnic composition of our population uniparentally inherited genetic markers were chosen.

Some Y-chromosome specific Single Nucleotide Polymorphisms (Snips) are considered ethnic-specific for Amerindian populations, such as the one located at the locus DYS199. Moreover, mitochondrial DNA sequencing of HVRI and the ins/del of 9bp in RV, might identify Haplogroups A, B, C, and D, that defines Amerindian matrilineage. Accordingly, the combined analysis of Y chromosome and mtDNA polymorphisms may provide a clue to address the ethnic composition of our heterogeneous population. A set of over 200 unrelated males inhabiting urban areas of some of the most populated provinces of Argentina including: Buenos Aires, Rio Negro, Mendoza, Santa Fe and Misiones were selected for analysis. All samples were typed for DYS199 and with the Charité´s Y-STR nonaplex. Nucleotide sequence of mtDNA HVRI was obtained in all cases in order to identify Amerindian mitochondrial haplogroups. The proportion of individuals with either Y and mitochondrial haplogroups not considered Amerindian (Caucasian?) was 47%, those with both Y-chromosome and mtDNA Amerindian haplogroups were 10% and the mestizo condition (either one or the other amerindian lineages) accounted for 43%. As depicted in most Latin American countries, there is in Argentina, a clear asymmetry regarding Amerindian matrilineage mestizo (83.3%) when compared with Amerindian patrilineage (16.7%). A clear discrepancy arose in comparison with the physical anthropological position that claims a minimal aboriginal component in Argentina's population. This contribution provides additional information that supports a more relevant Amerindian component in a country that claimed to be the most European one of the entire Latin America.

Y-chromosome SNP and STR analysis in a Belgian population sample

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Analysis of Single Nucleotide Polymorphisms (SNPs) is becoming a routine method not only in population and clinical genetics but also in the forensic field. SNPs on the Y chromosome are particularly of interest in paternity testing and forensic DNA-analysis especially in sexual assault cases. SNPs could also be helpful in those cases where the DNA is too degraded for STR-typing. We have developed a Y-SNP-assay based on multiplex-PCR of up to ten Y-SNPs followed by primer-extension (SNapShot, Applied Biosystems) analysis for identification of the polymorphism on an ABI3100 Genetic Analyzer (Applied Biosystems). 27 SNPs were selected from the Y Chromosome Consensus tree and divided into 3 multiplex reactions each with an amplicon length between 73 and 156 bp. The 27 SNPs define in total 27 haplogroups or haplogroupclusters. The first 10-plex allows identification of the major haplogroups while the other multiplex reactions can be used for further subtyping. This way, only 2 out of 3 multiplexes are necessary to define the haplogroup. The developed approach has been applied to a Belgian population sample of 98 males that has been previously typed with the Powerplex Y System (Promega). In total, 6 haplogroups (E3b, G, I, J2, R1a1 and R1b) were observed in more than one individual while 2 haplogroups (A and F*(xG,H,I,J,K) or J*) were each present in a single individual. The two most common haplogroups were R1b (60.6%) and I (13.1%) which is similar to other studies of West-European populations. These haplogroups were further investigated with the Powerplex Y System. Haplogroup R1b showed 45 different minimal haplotypes while haplogroup I contained 9 different haplotypes. The mismatch distribution for R1b was uni-modal and the phylogenetic network constructed with the minimal haplotype (excluding DYS385) showed a starlike structure. In contrast, the haplogroup I STR- haplotypes showed a tri-modal mismatch distribution. This tri-modal distribution could be an indication of the presence of 3 sub-clades in the population group belonging to haplogroup I. Further SNP-subtyping is necessary in order to prove this hypothesis. Finally, we identified a micro-variant (14.1) for DYS19 in the sample of 98 males and characterized this variant as a nucleotide insertion in the flanking sequences. The STR-haplotype of this sample was identical to another haplotype, except for DYS19. SNP-analysis showed now that they both belong to haplogroup J therefore proving that the micro-variant has been derived from this “ancestral” haplotype. This “ancestral” haplotype was also the most common minimal STR-haplotype in haplogroup J.

Y-STR haplotyping in seven Hungarian (speaking) populations

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Forensic genetic analysis for the nine Y-STR minimal haplotype loci (DYS19, DYS389-I/II, DYS390, DYS391, DYS392, DYS393, DYS385-I/II) was performed in seven Hungarian (speaking) populations. The sample collected from the mixed Budapest population was chosen as our reference population sample. Other four samples were also collected in Hungary from two Romany (Gypsy) populations of southwestern (Baranya county) and eastern (Debrecen region) Hungary, from Ashkenazi Jews residing in Budapest, and from Kiskun ('Small Cumanian') people living in a small village Fülöpszállás. In addition, two Hungarian speaking populations (Székely and Csángó) were surveyed in Transylvania, Romania. Samples collected in Csíkszereda (Miercurea Ciuc) and in Gyimesfelsõfok (Lunca de Sus) were to represent the Székely ('Sekler') and the Csángó populations, respectively.

In the population survey six variant allele types were detected in 12 males at the locus DYS385. Eight individuals harbouring the DYS385 *.-1 microvariant allele type had very similar Y-STR haplotype and at least seven persons belonged the same Y haplogroup. These findings may imply a recent common paternal ancestry of these Y chromosomes. The eight individuals having the DYS385 *.-1 microvariant allele type originated from the populations of Budapest (both reference and Jews), Baranya Romanies and Kiskun. A possible admixture may, therefore, be supposed for the males in these populations.

The diversity value for the nine-locus Y-STR haplotypes of the pooled population sample (474 males) was 0.994. The haplotype diversity values for the Romany population samples were smaller than those found in the other five populations. Of the identified 291 different Y-STR haplotypes, 225 proved to be unique. 41 haplotypes from 177 males were shared by two or more populations. The two most dominant haplotypes were shared by 21 and 13 Romany males, respectively.

By computing inter-population variance (Φ_{ST}) of Analysis of Molecular Variance (AMOVA) for the 7-locus Y-STR haplotypes there was no significant genetic correlation between the Romany population samples. Both the Romany samples, however, showed significant genetic distance from all of the populations, with the exception of the Jewish sample. There was no evidence to suggest significant genetic correlation between the Budapest reference and Ashkenazi population database as well as between Kiskun and Ashkenazi population. Our survey supports the previous findings of Thomas et al. (2002) that the paternally inherited Y chromosomes of Jewish males showed diversity similar to that of neighbouring populations and showed no evidence of founder effects. With pairwise comparisons of inter-population AMOVA, the Székely and Csángó populations could be clearly distinguished. Interestingly, these two populations from Romania showed no significant genetic distance from the Budapest reference sample.

Reference

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Analysis of the POLG CAG repeat length variability in North Eurasian populations

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The last decade reveals a rapid progress in the investigation of population variability of the maternally inherited mitochondrial DNA (mtDNA). The accumulation of mutations in human mtDNA was suggestive of a defect of nuclear genes responsible for mtDNA replication and maintenance. Among them, the mitochondrial DNA polymerase gamma (POLG; MIM# 174763) has been identified as an enzyme directly involved in induction of mtDNA mutations. We have investigated the frequency of different repeat-length alleles of a trinucleotide CAG microsatellite repeat within the coding sequence of the nuclear gene for the catalytic subunit of mitochondrial DNA polymerase gamma (POLG) in 12 ethnic groups of North Eurasia. The population sample was represented by 1330 individuals from three large geographic areas: Europe, Southwest Asia and Siberia/East Asia. We have found that 10-repeat allele of the POLG gene is the most frequent in all populations studied, being encountered at a frequency of 88-96% in regional groups. The heterozygosity level ranges from 22% in Europe and 13.6% in Southwest Asia to its lowest value of 7.4% in Siberia/East Asia. The present study provides evidence for a clinal distribution of the POLG gene heterozygosity in North Eurasian populations.

GEP-ISFG collaborative studies on Y chromosome STR loci: methods, population data and mutation rates

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GEP-ISFG (The Spanish-Portuguese Working Group of the International Society for Forensic Genetics)

The Spanish-Portuguese Working Group of the ISFG (GEP-ISFG) has included the use of Y chromosome STR markers in the yearly collaborative exercises (from 2001 onwards). The first exercise aimed predominantly at the improvement of methods while the second encouraged labs to gather population data, particularly for non-European populations. The corresponding results have been published [FSI 135:158(2003) and FSI 135:150(2003)]. In the last year, the study was organized in order to estimate mutation rates for the following Y STRs: DYS19, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA A10, GATA C4, GATA H4. In this study, 19 laboratories participated and a total of 1840 father/son pairs were analysed for 8 to 17 Y STR markers.
Among 22449 allele transfers, 50 mutations were observed, one at DYS389 I, DYS438 and DYS461; two at DYS390, DYS392, DYS393 and DYS437; three at DYS389 II and GATA H4; four at DYS19, DYS385, GATA A10, DYS460 and GATA C4; five at DYS391 and; eight at DYS439. A number between 974 and 2861 allelic transmissions were studied per locus and mutation rate estimates varied from 0.6959×10^{-3} (DYS389 I) to 6.3291×10^{-3} (DYS439). The overall mutation rate across the 17 loci was 2.2273×10^{-3} per locus/meiosis (95% CI: 1.273×10^{-3} – 2.426×10^{-3}).
Except in one case (DYS438), all mutations were single-step. For DYS438 a four-step mutation was observed, never reported before, in which allele 10 mutated to six repeats. We did not find mutations in more than one locus for the same father/son pair.

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The Romanov "controversy" and other issues in bone DNA typing.

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A recent study by Alec Knight and colleagues, purporting to expose "Molecular, forensic and haplotypic inconsistencies regarding the identity of the Ekaterinburg remain" was highlighted in Science ("Buried, recovered, lost again? The Romanovs may never rest", 6 Feb). Their reference sample for Tsarina Alexandra, a shrivelled finger believed to be of the tsarina's sister Elisabeth, yielded a mitochondrial DNA sequence that did not match that of Prince Philip, a known relative of both sisters.
Knight and colleagues assert that the Ekaterinburg bones, identified as those of the Romanovs by Gill et al., were in fact contaminated. They claim that the molecular behaviour of the Ekaterinburg bone DNAs reported by Gill et al. is "wholly inconsistent with the behaviours of degraded DNA" reported elsewhere, and suggest that the Gill et al. study does not conform to new rigorous standards for ancient DNA research. However, we show that the alleged scientific and historical evidence presented by Knight and colleagues is questionable. In addition, we present empirical evidence to challenge the so-called "criteria of authenticity" for ancient DNA sequences.

Reference

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Use of Y-STR haplotyping and large population databases to predict the population-origin of unknown male DNA

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In August 2002 the remains of a badly burnt man was found within Swedish territory, a few kilometres from the Norwegian border. Due to his post mortem body state it was not possible to determine his population affiliation. However, his dental status clearly showed that he could not be of Swedish origin.
Independent investigations by three different forensic disciplines, anthropology, odontology and genetics, reached the same conclusion that this male corpse might have an Asian population origin. His shovel-shaped teeth, a facial reconstruction and the shape of his hair all indicate a Mongolian/Asian origin.
In genetics, we analysed the Y-chromosomal STR-based haplotype profile for a prediction of his population-origin. This is a promising approach since the neutral Y-chromosomal markers are probably the best-known ancestry informative markers (AIM), known so far. They are confined to a patrilinear haplotype transmission and are also susceptible to genetic drift.
The unidentified man's Y-STR-haplotype: 15,14,31,24,10,13,12,16-20 (for nomenclature see `www. yhrd.org`) had no matches in a set of 26 654 minimal haplotypes from 236 populations (YHRD release 14, September 18th, 2004). DYS385 16-20 was not found in the worldwide database and further search was thus conducted without this locus. Still no matches were found. However, a search for closely related 1-mutative step “neighbouring" haplotypes revealed 35 matches. Of these 15 were found amongst 21 636 haplotypes from Eurasian metapopulations and 19 amongst 2 911 from East Asian metapopulations and 1 amongst 1 624 from African metapopulations, using the PopSearch option. The GeoSearch option gave 9 matches amongst 16 779 European haplotypes and 22 amongst 5 315 Asian. One match was also found in North America and three in Latin America.
Based on this neighbour search, using PopSearch, a Bayesian calculation gave a ratio of probabilities of 9:3:2:1 that this Y-chromosome originates in metapopulations from East Asia: some other: Africa or Eurasia. By GeoSearch the ratio obtained was 7:3:2:2.1 for Asia:Latin America:North America:some other or Europe.
The reliability of this approach was also tested for by searching the Y-STR-haplotype database for haplotypes of a few selected males with known population origin.

Improving the sensitivity and specificity of PCR amplification for forensic Y-STR typing

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The polymerase chain reaction (PCR) is a technique that amplifies a target DNA sequence in vitro and is one of the most widely used techniques of molecular biology. For Y-STR typing by fragment analysis, PCR is the most basic and important technique, and we cannot proceed further without PCR amplification products or amplicons. Problem with PCR sensitivity does arise in forensic DNA typing, especially when dealing with poor quality or low-copy number nucleic acid templates. Many variations of the basic procedure have been described and applied to solve this problem and they include nested PCR, ultraviolet-mediated DNA cross linking to destroy exogenous template, etc. These methods are partially effective for they do not increase remarkably PCR sensitivity and specificity.

With the intention of designing a more effective and simple method for high resolution of PCR, we investigated and improved various steps of PCR, including sample collection, template preparation, pre-PCR procedure, and primer selection using Y-STR loci, especially, DYS19 and DYS393 typing. Here we report PCR amplification of microdissected DNA, newly developed modification of PCR, and direct sequencing applied for practical cases in rape and murder. These techniques are simple, economic, time saving, and applicable to criminal investigation and ancient DNA analysis, by using any type of PCR with poor quality or low-copy number nucleic acid templates. In addition, curious amplification found in DYS393 typing will be also exhibited in the presentation.

Y-STR haplotypes from East Germany – differences between carriers of surnames of Germanic and Slavic origin?

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Despite its name, which seems to imply an utterly Germanic origin, Germany has always been a gateway for migration. This includes the arrival of eastern Germanic tribes and Slavs, driven by the pressure of the Huns, which eventually led to the downfall of the Roman empire. As a consequence of these population movements, the area which today forms East Germany was inhabited by Slavic people in the early middle ages. Whilst most Slavic tribes (like the Prussians) completely abandoned their language during the following centuries and turned "typically German", the Sorb minority in the Lausitz region maintained much of its cultural identity and today represents the only people speaking a Slavic language in Germany. What has been left from the Slavic languages in most parts of East Germany, however, are the names of cities and some family names. In addition, parts of East Germany such as the South of Saxony-Anhalt are traditional mining and industrial areas which experienced significant migration of Slavic workers during the industrial revolution. Both waves of Slavic migration could have had impact on the composition of the local population and are documented by a high percentage of surnames with Slavic origin. The goal of the present study was to investigate whether Y-chromosome haplotype analysis is capable to distinguish groups with surnames of different origin. To this end, DNA samples were obtained from 400 males born in the south of Saxony-Anhalt. Samples were divided into three groups namely those with a Germanic surname, those with a Slavic surname and those with mixed origin of the surnames. The minimal Y-STR haplotype of these two groups was analysed by AMOVA. A highly significant difference ($p < 0.001, F_{ST} = 0.0309$) between the Germanic and the Slavic groups was observed. When comparing this to other populations using published data, this difference is similar to that between European populations of large geographical and linguistical differences (like e.g. between Cologne and Budapest). On the other hand, the group with surnames of the mixed origin were indistinguishable from the Germanic group ($F_{ST} = 0.0008$).
Our results reflect that the Y chromosomal lineages of Germans born in southern Saxony-Anhalt differ depending on the ethnic and linguistic origin of their surname.

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Demonstration of the usefulness of Y-chromosomal loci for casework – a serial rape case was solved

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In the years 2000-2001 at least four rape cases (four different female victims in four different flats in Magdeburg) happened showing the following characteristics:

- The offender entered the ground floor of the victim through the open window.
- The offender blindfolded the victims and threatened them with a knife.
- The offender forced the victims to hand over their EC cards including PIN.

	DYS19	DYS385I/II	DYS389I/II	DYS390	DYS393
1. Case Apple (from the garden)	16	14/14	13/32	24	13
Cup (from the flat of the victim)	16	14/14	13/32	24	13
2. Case Swab from the hand of the victim	16	14/14	13/32	24	13
3. Case Sperm on the victim‘s nightdress	16	14/14	13/32	24	13
4. Case Bottle of ice tea at the bedside of the victim	16	14/14	13/32	24	13

Table: *Use of male specific systems (DYS systems) concerning traces from four rapes*

The results in the table show that the four rapes could be carried out by one and the same male person. The results were confirmed with all (autosomal) STR systems from the SGM Plus kit plus SE33.

In January of 2004 the Inferior Court of Magdeburg gave the order for the DNA-analysis of the saliva sample of a criminal. A hit was obtained with the mentioned traces of the described four rapes in the German DNA Database.

Some stages of the criminal career of the offender

- in August of 1997 the offender was convicted of theft in 14 cases and of computer fraud in 6 cases to imprisonment of two years and six months.
- in January of 2000 he was released and put on probation.
- in January of 2004 the order was given to analyse his saliva sample.
- in August of 2004 the defendant was given a prison sentence to 11.5 years.

Survey of Y-chromosomal Microsatellites in Human and Non-Human Primates

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By in silico screening of the near-complete DNA sequence of the human Y chromosome and subsequent experimental testing we identified 166 useful new human male-specific microsatellites, which increases the number of known human Y microsatellites from previously 53 to overall 213. Using sequence and polymorphism data we studied the factors influencing Y microsatellite variance and revealed that for simple loci, the average repeat count accounted for the highest proportion of their repeat variance ($\sim$ 34%) whereas for complex loci, the largest proportion of the variance (again $\sim$ 34%) was explained by the average repeat count of the longest homogeneous array, which normally is variable. In these complex microsatellites, the additional repeats outside the longest homogeneous array significantly increased the variance, but this was lower than the variance of a simple microsatellite with the same total repeat count. In addition, we analysed 136 of the newly-identified human Y-chromosomal microsatellites in five (sub)species of non-human primates. We identified 83 male-specific loci for chimpanzees, 67 for gorillas, 45 for orangutans and 19 loci for mandrills. Success in male-specific amplification of human loci in non-human primates was significantly negatively correlated with divergence time from the human lineage. There were significantly more male-specific loci with longer alleles in humans than with longer alleles in the non-human primates; however, this significant difference disappeared when only the loci which are polymorphic in non-human primates were analyzed, suggesting that ascertainment bias is responsible. As a result of this work, a large number of new, highly polymorphic Y-chromosomal microsatellites are now available for population genetic, evolutionary, genealogical, and forensic investigations as well for primate genetic and behavioural ecology studies.

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Is there a reliable Y-STR database for you?

P de Knijff

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No abstract available.

Forensic Interpretation of Y-Chromosomal DNA Mixtures

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The mathematical concept previously introduced for the forensic interpretation of DNA mixtures using non-associated genetic markers has been adapted to the assessment of haplotypes. Such calculus is required, for example, when Y-chromosomal markers are used in forensics. In addition to outlining the general mathematical framework, we devise two approaches to its practical computational implementation, involving either the inclusion-exclusion principle of probability theory or a recursion in the number of unknown contributors invoked. The two approaches scale differently, depending upon the complexity of the case and the diversity of the markers used. The performance of Y-chromosomal microsatellites (Y-STRs) as a means of trace donor discrimination has been assessed by simulation, using the derived formulas. Based upon data from the Y-chromosomal Haplotype Reference Database (YHRD), the exclusion chance of a non-contributor is shown to vary between 95% in the case of two contributors, and 70% for five contributors. With only one additional contributor, half of all contributing suspects would yield a log-likelihood ratio in favour of donorship of 1.61 or higher, although the median drops to 0.66 with four additional contributors. It must be emphasised that these estimates of the discriminatory power of Y-STRs are likely to be conservative since the simulations involved only haplotypes known to occur in YHRD.

The genetic landscape of Europe as revealed by analysing the European Y-STR haplotype distribution

M Krawczak on behalf of the Forensic Y-Chromosome Research Group

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Previous studies of human Y-chromosomal single nucleotide polymorphisms (Y- SNPs) established a link between the extant Y-SNP haplogroup distribution and the prehistoric demography of Europe. In contrast, our analysis of seven rapidly evolving Y-chromosomal short tandem repeat loci (Y-STRs) in over 12,700 samples from 91 different locations in Europe reveals a signature of more recent historic events, not previously detected by other genetic markers. Cluster analysis based upon molecular variance yields two clearly identifiable sub-clusters of Western and Eastern European Y-STR haplotypes, and a diverse transition zone in central Europe where haplotype spectra change more rapidly along longitude than latitude. This and other observed patterns of Y-STR similarity may plausibly be related to particular historic incidents including, for example, the expansion of the Franconian and Ottoman Empires. We therefore conclude that Y-STRs are capable of resolving male genealogies to an unparalleled degree and may therefore represent a useful means to study local population structuring and recent demographic history.

The marker DYS464 – results of studies in five populations

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The Y-STRs are well established in the forensic routine case work. To improve typing with so called minimal and extended core sets additional loci have been developed and validated. Introduced and described by Reed et al. (2002) DYS 464 is considered as the most polymorphic Y-STR. To evaluate its forensic value we analyze five populations- Saxonia, Estonia, Lithuania, Latvia and Poland. The extended core set and Y-SNPs were additionally analyzed. Our results are in agreement with previous foundings that DYS464 is a highly polymorphic marker. The power of discrimination (PD) obtained is 0.90 up to 0.94. Combining the marker with the minimal or extended core set the increase of the discrimination power is not significant. When Y-haplogroups were considered in calculations the results look different. We showed that DYS 464 has a high discrimination index for detection of haplogoup and this way give additional information. However, the genetic background of the marker remains unclear. Lack of the possibility of amplification each copy of DYS 464 individually causes uncertainty with accessing and matching number of alleles and number of copies. In our study profiles with up to five alleles were observed. Nevertheless, we can conclude that DYS464 is a highly polymorphic marker with a high potential of haplogroup discrimination.

GEP-ISFG mitochondrial DNA collaborative exercise 2004: assessing mtDNA male-female contribution in a semen/saliva mixture DNA sample.

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As part of the 2004 GEP-ISFG mtDNA proficiency exercise, the Quality Control Unit of the National Institute of Toxicology and Forensic Sciences (Madrid) provided a mixture stain (called M6) consisting of 100 (L of saliva from a supposed female victim and 50 (L of a 1:20 semen dilution from a supposed

offender. Blood samples of both donors were also provided (namely, M4 and M5 from victim and suspect, respectively). HVS-1/2 haplotypes were 263G 315.1c for M4 and 16266T 263G 309.1C 315.1C for M5. MtDNA sequencing analysis of the saliva/semen mixture M6 produced an unexpected consensus result (13 out 19 labs): only the HVS1/2 saliva haplotype (M4) was detected, either after preferential lysis or after complete DNA digestion. This result paradoxically contradicted the autosomal STR profile obtained from a complete lysis, where the male component (M5) was predominant.
Several labs carried out additional experiments to clarify the puzzle: (i) two labs analysed HVS-1 with additional different primer sets which yielded the same unique M6 haplotype, thus allowing rejection of the hypothesis of a primer binding site mutation in the semen of the donor; (ii) other lab reproduced the same mixture experiment using different saliva and semen donors, an experiment that yielded the same pattern as in M6: only the saliva haplotype was detected; (iii) the same lab performed HVS-1 amplifications using decreasing nuclear DNA dilutions from semen and saliva and the results showed a loss of signal at 2 pg of nuclear DNA from semen, while at 0.2 pg of nuclear DNA from saliva amplicons were still detected; (iv) theoretical calculations of the relative number of mitochondrial DNA copies in spermatozoa and epithelial cells in the mixture was made by another lab, and (v) finally, one lab carried out coding region SNPs analysis using SNaPshot, a more sensitive method than sequencing that allowed the detection of the two components (M4 and M5) of the M6 sample. In conclusion, the results pointed to the existence of different relative amounts of nuclear and mitochondrial DNAs in saliva and semen. This circumstance could deeply influence the detection of mtDNA in evidences with unbalanced mixtures of different fluids and could lead to false exclusions.

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Development and validation of a highly discriminatory 17-plex Y-STR PCR amplification assay

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Current interpretation of sample mixtures that are analyzed with autosomal markers via PCR can suffer from competition between a relatively small amount of male DNA and a large amount of female DNA. These specimens require the forensic analyst to make a judgment call on which autosomal STR loci correspond to the major and minor contributor. A multiplex PCR amplification system targeting Y-specific loci yield a high degree of confidence that only the male contributor is being analyzed. We are currently in development and validation of a 17-plex Y-STR system with an increased discriminatory capacity over current commercial Y-STR assays. The Y-STR multiplex includes all the loci in the "European minimal haplotype"; DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and the SWGDAM recommended Y-STR loci; DYS438, DYS439. Additionally, we have included six highly polymorphic loci DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4. To ensure no overlap between allele ranges, loci were labeled with 6-FAM™, VIC™, NED™, and PET™ dyes. We have also generated an extensive allelic ladder containing 137 alleles. The multiplex amplification conditions were optimized on the Gene Amp PCR system 9600 and 9700 thermal cyclers. The amplified products are separated on Applied Biosystems Genetic Analyzer instruments that support G5 matrix analysis such as the ABI PRISM® 3100, 3100-Avant and 310 Genetic Analyzers.

Validation studies demonstrating the effectiveness of the Y-STR multiplex will be presented including male to female, male to male, sensitivity and species specificity studies.

The Y-STR PCR amplification system described, used in conjunction with the Quantifiler™ Y Human Male DNA Quantification Kit, produce reliable and accurate Y haplotypes from the types of samples seen in the forensic laboratory. The system can be used in conjunction with a number of Applied Biosystems Thermal Cyclers and ABI PRISM® Genetic Analysers, and provides the forensic scientist with a complete set of tools for Y chromosome analysis.

EMPOP – A new mtDNA database for forensic purposes

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The mitochondrial genome and its control region (CR) in particular perfectly lend itself for the discrimination of forensic samples that do not contain enough high quality DNA for standard STR-typing and/or involve a case scenario, in which the unimodal inheritance along the maternal line provides useful information. Given an exact mtDNA sequence match between a crime stain and a suspect, it is of interest to determine the frequency of the sequence in the population in order to calculate the probability of a chance match. Generally, the expected frequency of an mtDNA haplotype is estimated by comparison to a collection of mtDNA haplotypes in a population database.
The quality of published mtDNA population data has been in the centre of criticism throughout the past four years. Performing a posteriori phylogenetic analysis and proficiency testing it has been demonstrated, that the compilation of mtDNA databases is prone to error, mainly caused by

- the complexity of the so far employed laboratory method (increased chance of sample mix-up),
- the sometimes misconducted laboratory procedure,
- the sensitivity of the methodology (increased risk of contamination) and
- the traditional manual way of data transcription

In order to become acquainted with the problematic patterns associated with mtDNA analysis, we scrutinized published mtDNA data and performed interlaboratory tests. We arranged the observed inconsistencies according to their cause and phenotype and tried to identify and employ appropriate methods in order to adopt a concept to minimize these in the future.
This concept was applied to the establishment of a web-based mtDNA population database for forensic purposes (EMPOP). In addition to making mtDNA population data available for frequency estimations, EMPOP serves as a platform for discussion

- by recommending sampling and typing strategies, in order to prevent sample bias,
- by introducing modern mtDNA CR typing strategies,
- by harmonizing and clarifying interpretational issues concerning the mtDNA nomenclature, and
- by presenting solutions of automated data handling, data transfer and data storage.

Most importantly, we see the need for closer collaboration and exchange of knowledge among the forensic institutes with respect to mtDNA databasing and casework issues.

Weathering Stormy Seas: Challenges and Progress in mtDNA Forensic Applications

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Observers following recent high profile publications and debates associated with mtDNA and its forensic applications might wonder if forensic mtDNA is in serious trouble. This presentation will briefly review some of these issues, such as errors in mtDNA databases, paternal inheritance, and recombination. It will also cover the "controversy" regarding the authenticity of the Romanov remains, where the closely related fields of forensic DNA and ancient DNA study have intersected in an interesting manner. Despite these recent challenges, which have various levels of operational significance and requirement for future awareness (ranging from essentially none to quite a lot), we can view forensic mtDNA applications as having a brighter future than ever. Enhanced systems for high throughput mtDNA population databasing, with automated laboratory and data handling will be discussed. Coupling this with improved database curation, access, and quality control monitoring, via the EMPOP database, will provide a vastly improved resource for global forensic population comparisons. We are also now at a stage where the entire mtDNA genome has become practically accessible, to greatly decrease one of the principle limitations of mtDNA testing: the low power of discrimination that occurs when common control region haplotypes are encountered. SNP assays are important developments in this regard, and can also be used for attributing samples to continental/haplogroup origin with greater certainty.

Expectancy of Y-STR haplotype diversity parameters under different population scenarios

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The issue of haplotype diversity is essential when dealing with databasing of haploid hypervariable loci, as in the case of Y-STRs (and hypervariable mtDNA sequences). This is due to the fact of being impossible to apply the product rule for frequency estimation. Roewer et al. (2000) (and further corrections in Krawczak, 2001) developed a method for haplotype frequency estimation based on a Bayesian approach, which is, nevertheless, dependent on the a priori haplotype distribution. We also ended up with the conclusion that the observed haplotype distribution strongly influences the levels of saturation extrapolated from empirical saturation curves. So, a basic question remains: to what extent the haplotypes deposited in the Y- STR Haplotype Reference Database really reflect the haplotype diversity of the various populations sampled?
We will present the results of computational simulations for evaluation of the sampling size effects on the expectancies of Y-STR haplotype diversity measures, under different population scenarios, namely: (1) when departing from equally frequent haplotype frequencies; or (2) from highly different haplotype frequencies (a scenario of differential reproductive success/selection or strong drift); and (3) incorporating sub-structure in the database (by mixing two substantial different datasets). We think that the saturation curve behaviour of this simulated database, in terms of number of haplotypes and haplotype molecular diversity, can help us to gauge the present status of the Y-STR database.

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Genetic differentiation follows political borders in Europe: Y-chromosome variation in Poland and Germany

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To test for human population substructure within Europe we have investigated Y chromosome diversity using seven microsatellites and ten binary markers in samples from eight regionally distributed populations from Poland ($n = 913$) and eleven from Germany ($n = 1215$). Based on both marker systems we observed statistically significant genetic differentiation between all Polish and all German regional populations but also genetic homogeneity within each of the two neighboring geographic regions. We suggest that this scenario can be best explained by very recent events in human population history, namely the politically forced human resettlements during and after World War II, rather than more historical population movements. In addition, our findings have important consequences for the use of Y chromosome markers in forensics and strongly argue in favor of regional Y chromosome databases and / or the implementation of population substructure into more global Y chromosome databases.

Evaluation of the "Minimal Haplotype standard" and of new highly discriminating STR loci on the Y chromosome

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The Minimal Haplotype (MH), definitely DYS19, DYS385, DYS389-I, DSY389-II, DYS390, DYS391, DYS392, and DYS393 was evaluated by the International Forensic Y-User Group as a standard for Y-chromosomal STR-typing.

We have developed a multiplex application for the Minimal Haplotype loci, named Mentype Argus Y-MH PCR Amplification Kit. The kit was developed for fast and reliable generation of male specific DNA profiles from mixtures of male and female DNA, detecting a minimum of 100 pg of male DNA in up to 600 ng female DNA. Validation studies have been performed and the results will be presented shortly.

In-between the complete DNA sequence of the Y chromosome was screened for microsatellites (Redd et al., 2002; Kayser et al., 2004). Out of the published loci, we selected those new STR showing excellent genetic diversities between 0.750 and 0.973, designed small multiplexes and generated population data for a reevaluation in a German population. We are looking forward to include those loci useful for highly discriminating Y chromosomal DNA typing in a new PCR Amplification Kit.

Assignment of the YHRD samples to metapopulations and resulting search options

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With release 14 from Sept. 2004 the YHRD has been updated and remodelled. According to the latest recommendations of the forensic genetics societies the error-prone dinucleotide marker YCAII has been replaced by the Y-STR loci DYS438 and DYS439 (SWGDAM core set). About 17% (or 4,548 haplotypes from 29 populations) of the overall 26,654 haplotypes in this release have already been typed for the new 11-loci YHRD core set: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385I/II, DYS438, DYS439. Moreover, to allow a frequency estimate in related population groups the single YHRD populations are now sorted by so-called metapopulation affiliation. For the search option "GeoSearch" the population samples uploaded to the YHRD have been grouped according to their continental affiliation. The seven geographically defined metapopulations are: Europe, Asia, Latin America, North America, Africa, Oceania/Australia, Arctic. For the search option "PopSearch" the population samples uploaded to the YHRD have been structured according to their ethnic affiliation (mostly linguistically defined, see Pennisi 2004). The seven linguistically- ethnically defined metapopulations are: Eurasian, East Asian, African, Amerindian, Australian Aboriginal, Eskimo-Aleut and Admixed. Three of these metapopulations are currently very large and consist of subpopulations:

Eurasian – European, Altaic, Caucasian, Uralic, Arabian, Indo-Irania, Indian.

East-Asian – Korean, Japanese, Sino-Tibetan, Austroasiatic, Thai, Austronesian, Indo-Pacific.

African – Subsaharan, Afro-Asiatic, Afro-American, Afro-Carribbean.

The resulting search options, including the "neighbour search", and reporting issues will be discussed.

Haploid markers in the Finns

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No abstract available.

New challenges in mitochondrial DNA profiling

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The arrival of new methodological improvements (automated sequencers, new sequence chemistries, etc.) to forensic labs, has not been shown to be efficient enough in reducing the high error rate recorded in the recent forensic and clinical literature. This characteristic clearly contrasts with the classical high level of precision of the forensic community with respect to other disciplines. Besides a more careful sequencing design, visual inspection of mtDNA profiles performed in a phylogenetic framework can help to reduce the error rate by an order of magnitude. This presentation outlines the phylogenetic approach and discusses topics such as mtDNA mutation rate heterogeneity and the weight of the evidence, ethnic affiliation of mtDNA profiles, and reference databases.

D-loop 3'$(CA)_n$ repeat heteroplasmy: Inheritance studies in two pedigrees

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The D-loop 3' $(CA)_n$ repeat is the only STR like polymorphism in the human mitochondria genome. We investigated two pedigrees which showed heteroplasmy in regard to this locus. One of the pedigrees investigated here exhibited heteroplasmy as L00309.2 C/T and $(CA)_4/(CA)_5$, as well. Whereas the L00309.2 C/T ratio was nearly concordant between three brothers and their two cousins the (CA)n heteroplasmy occurred only in one branch of the pedigree. The $(CA)_4/(CA)_5$ proportion between the brothers was rather different. Unfortunately, none of the pedigree members of the maternal and grandmaternal generation were available for investigation. The second pedigree consisting of three sisters and their children showed a $(CA)_5/(CA)_6$ heteroplasmy. Again, the ratio of the heteroplasmy components was rather different between the sisters. The findings suggest that $(CA)_n$ repeat heteroplasmy inheritance may be influenced by a narrow bottleneck.

Interpreting Y-chromosomal DNA variation

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The determination of the near-complete sequence of the euchromatic portion of the Y chromosome of one man (Skaletsky et al., 2003, *Nature* **423**, 825- 837) and the availability of more efficient methods for identifying variants in the Y chromosomes of others are leading to a better understanding of the extent of Y-chromosomal DNA variation. Differences occur at all scales from single nucleotide polymorphisms (SNPs) to major chromosomal rearrangements. The most useful for forensic and evolutionary purposes are SNPs and STRs, but copy-number polymorphisms (CNPs) must also be taken into account. Around 300 SNPs have been incorporated into a phylogeny and haplogroup nomenclature maintained by the Y Chromosome Consortium (`http://ycc.biosci.arizona.edu/`), but there are very many more potentially useful Y-SNPs. A recent survey (Kayser et al., 2004, *Am. J. Hum. Genet.* **74**, 1183-1197) has brought the number of useful polymorphic Y- STRs (excluding dinucleotides) up to almost 200, and is likely to include most of those available. CNPs resulting from large-scale duplications and deletions affect regions such as AZFc, and, along with gene conversions, can complicate the simple pattern of variation expected on a haploid chromosome. I will discuss the information available about these different classes of markers and typing methods.

Y-chromosomal variation shows a high degree of geographical structure, usually explained by its low effective population size and sensitivity to genetic drift, and possibly the effect of patrilocality. Another consequence of these influences is that Y variation is relatively easily reshaped by historical events. In this part I will discuss methods for analyzing spatial patterns of variation and inferring time depths, emphasizing their strengths and limitations. Data from East Asia (see Xue et al., this meeting) will be used as an illustration.

Y-chromosomal DNA variation in East Asia

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East Asia has been inhabited by modern humans for $> 50,000$ years and now holds more than one quarter of the world population. Genetic analysis using classical markers identified a major north-south distinction (Cavalli- Sforza et al. 1994), which may reflect separate origins of northern and southern populations from different migrations out of Africa. The Y chromosome provides high-resolution male haplotypes and thus an opportunity to investigate male-specific history. We have typed 1012 individuals from 28 populations in China, Mongolia, Korea and Japan with 45 binary markers (mostly SNPs) and 16 microsatellites from the Y chromosome. SAMOVA analysis of Y-SNPs was used to identify the geographical divisions that apportion the maximum amount of genetic variation between groups (Dupanloup et al. 2002). It did not show the traditional north-south division, but identified small groups of distinct populations, mainly in the south. Why should Y-chromosomal variation show a different structure from that of the rest of the genome? We have investigated the distribution and time- depth of individual haplogroups. For example, haplogroup O is largely confined to East Asia and one of its subdivisions, O3, is widespread and common in China. A subset of these chromosomes, O3/-d, is concentrated in southern China, particularly in the two Yao populations which formed one of the major SAMOVA divisions. The time depth of the O3/-d lineage was estimated at approximately 5,900 (4,500 - 8,200) years from its microsatellite variation using the program BATWING (Wilson and Balding 1998), so must have spread after this date. A second common haplogroup is C, and C3c chromosomes are concentrated in northeastern China where they are present at highest frequency in the Oroqen population, another of the major SAMOVA divisions. A substantial subset of them appear to have expanded very recently, < 900 years ago. These results suggest that East Asian Y-chromosomal variation has been substantially reshaped by recent events within Neolithic and historical times.

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Y-STR differentiation and substructuring in Pyrenean populations.

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The Pyrenees are a mountain range which spans 430 km from the Atlantic to the Mediterranean shore. Its widest section (160 km) lies in the central part and contains also the highest peaks, most over 3,000 meters. Though the Pyrenees have been populated since Paleolithic times, they present a very difficult geography that produces some degree of isolation of populations. Due to this orography, Pyrenees were perhaps a marginal area for the advance of the farming wave during Neolithic (Arias Cabal, 1991) and could be less affected by the farming spred than the rest of the Iberian Peninsula.

We studied the 9 loci comprised within the minimal haplotype of the Y-STR Haplotype Reference Database (`www.yhrd.org`) in the autochtonous populations from East Pyrenees – Cerdanya ($n = 42$) and Urgell ($n = 34$) – Central Pyrenees – Aran ($n = 30$) –, and West-Pyrenees – Jacetania ($n = 28$) and Lesaca ($n = 42$). Nine other Iberian populations from Spain and Portugal were used for comparison. Two Basque samples were also introduced in order to comprise the Basque- speaking region within the study. Genetic diversity parameters, AMOVA and mismatch distributions reveal substructuring of Pyrenean populations according to a East-West gradient. Results also point out a certain degree of differentiation between Pyreneans and the rest of Iberian samples. The genetic landscape produced may well support the isolation of the Pyrenean populations during the Neolithic diffusion due to the geography of the region.

Validation of the Y-STR markers that make up the "European Minimal Haplotype"

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Y-chromosome short tandem repeats can provide valuable information for cases of sexual assault and questioned paternity.

The aim of the present work was to validate a multiplex polymerase chain reaction (PCR) capable of simultaneously amplifying all the Y-STR markers that make up the "European Minimal Haplotype" (DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393). Following optimisation of the PCR, male/male and male/female mixtures were analysed. This study demonstrated that the male profile is discernible in a mixed stain in which the male DNA comprises 1/50 of the total. For the male:male samples a complete haplotype was detected for both males at a ratio of 1 : 20 and 20 : 1.

A study on Y-STR haplotypes in the Saxon population from Transylvania – is there evidence for a German origin?

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Y chromosome markers are increasingly used to investigate human population histories, being considered to be sensitive systems for detecting the population movements. In this study we present Y-STR data for Transylvanian Saxons in comparison with Y-haplotypes from Romanians and other European populations. The Transylvanian Saxons, called like that since medieval times, are representing a Romanian minority population with assumed German origin. They have settled in the Arch of Romanian Carpathian Mountains in the earliest of the 12th century. Historical and dialectal studies strongly suggest that they do not originate from Saxony, but more probably from the Mosel riversides (Rhine affluent) and also from the Eifel Mountains Valley (present territory of Luxembourg). Living protected by fortified cities in compact communities, they still represent a quite distinct population in Transylvania. Males selected for this study had all Saxon surnames and were classified by the birthplace of the paternal grandfather. The typing results reflect high Saxon population haplotype diversity. Furthermore, we present data on the haplotype sharing of the Saxon population with other European populations, especially with Germans as well as with Romanians and Transylvanian Hungarians.

Y-STR typing of an Austrian population sample using a 17-locus multiplex PCR assay

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Y-STR haplotypes were determined from a sample of 135 unrelated males and 70 sons from Tirol (Austria) using the AmpFlSTR® Yfiler™ PCR Amplification Kit (Applied Biosystems) that coamplifies 17 Y-STRs. The loci are distributed among four fluorescent dyes and range between 100 and 326 bp. The panel of markers includes the 9-locus European minimal haplotype and the markers DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4. A total of 130 different haplotypes (125 were unique) were identified by the 17 Y-STR markers, meaning an increase of 20 compared to the minHT. The number of alleles and haplotypes, the gene diversities and the cumulative haplotype diversity are compared with results obtained with the minHT-loci only. Additionally the STR data are discussed in connection with the Y-SNP haplogroup structure.

Segregation of heteroplasmic length variants in HVI region of mitochondrial DNA by size-based separation method

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Length heteroplasmy in HVI mitochondrial DNA (mtDNA) region was investigated from maternally related family members with a T16189C transition to determine length variants distribution performing a quantitative and qualitative analysis using size-based separation of fluorescently-labelled polymerase chain reaction products by capillary electrophoresis. This method reveals the relative proportions of length variants in individuals with this homopolimeric tract represented by multiple peaks of different size and heights. The results shows different profiles of length heteroplasmy mtDNAs from buccal swab of related family members and peak patterns resulted up to 6 length variants in some individuals. Although genetic characteristics of length heteroplasmy remain to be determined, this study could provide more information about mtDNA heteroplasmy and mitotypes segregation in favour of the hypothesis of a narrow bottleneck.

Mitochondrial DNA diversity in Nepal: preliminary analysis of the first hypervariable segment, HVS-1

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The greater Himalayan region is the most ethno-linguistically complex area of the Eurasian continent. Its intricate mosaic of languages suggests that the topography of the Himalayan mountain range has shaped and channeled prehistoric population movements. If so, it is also likely to have influenced genetic variation in the region, and an analysis of the molecular genetics of modern Himalayan groups, together with parallel studies of their languages, should provide insights into the prehistory of the region. Our collaborators have collected DNA samples from 954 individuals from Nepal and 1051 individuals from Bhutan, representing 12 and 19 main groups, respectively, which were identified on the basis of language, ethnicity and geography. Members of the collaboration are studying markers throughout the genome, including microsatellites and single nucleotide polymorphisms on the autosomes, Y chromosome and mitochondrial DNA (mtDNA). The objective of the present work is to characterize the genetic diversity of the 12 population groups in Nepal by analyzing the sequence variability of the HVS-I control region of the mtDNA. A 1.2 kb fragment was amplified in all individuals (771 males and 183 females) using the primers L-15897 and H-580, and then purified using the Exo-I-SAP method. The 341 bp HVS-1 sequences are currently being sequenced directly from the PCR products using the primers L15996 and H16401. We will be assessing the extent of the mtDNA diversity in Nepal as well the geographic distribution of such genetic variation. Our genetic data will be then compared to the linguistic diversity and the distribution of languages to test models of the peopling of the greater Himalayan region that have been put forward by linguists and archaeologists.

Collaborators:
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Verification of the lineage on the basis of Y-STR haplotypes

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A man reported to our Department with his genealogical tree reconstructed from 1642 on the basis of archival sources. However, he did not find such materials concerning 11 men having the same name. After receiving their consent, he decided to verify their lineage using genetic criteria. Our task was to determine the relationship between our client and those men. The cheek swabs were used to determine DNA polymorphism type Y- STR. Haplotypes were defined by the amplification technique in the range of the following loci: DYS19, DYS891, DYS38911, DYS390, DYS391, DYS392, DYS393 and named according to the terminology recommended by the International Society of Forensic Genetics. The frequency of occurrence of the haplotypes found, which is necessary for such calculations, was obtained on the basis of the available internet site: www.ystr.org of the European database. Using these data the family affiliation was confirmed in 4 and excluded in 6 men. In one man the earlier unidentified allele 10.2 was found in DYS385, which was shorter than the “family” allele 11 by two nucleotides and therefore his family affiliation was not excluded as the difference observed could have resulted from the deletion within the allele 11.

First report of mitochondrial DNA CR variations in the Hungarian population

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We present here a new mtDNA database of 225 unrelated European "Caucasians" from Hungary, a European population that has not been previously studied. To assess the mitochondrial DNA polymorphisms of this population, a reference population sample was collected from genetically unrelated persons of the mixed (heterogeneous) population of the capital Budapest. For this database, sequence data generation was highly redundant for two purposes: 1) to minimize any potential errors in the sequence evaluation and 2) to validate the mtDNA sequence analyses at the Hungarian Police Laboratory (HPL) in an interlaboratory Quality Control measure. The sequences were independently generated for HV1 and HV2 only at the HPL, and for the entire control region at the Armed Forces DNA Identification Laboratory (AFDIL). As a result of this project, an initial Hungarian reference population database was established which could be used for interpreting the weight of mitochondrial DNA evidence presented at Hungarian court.

Entire control region sequencing was performed by a completely automated process of laboratory manipulations (including robotics for each step of amplification and sequencing) and data export which did not involve any manual transcription. For almost all of the samples, the entire control region approach involved a single amplification, reducing the potential for "phantom recombination" to near zero. The data produced from the entire control region by the AFDIL were compared to that performed manually for HV1/HV2 in Budapest as a further test for database correctness and to validate all phases of mtDNA sequencing methods in the Hungarian laboratory.

The random match potential and pairwise comparisons within the data and with that of neighbouring countries like Austria and other European databases is reported. The exclusion potential of the entire control region versus HV1/HV2 only will be compared with the conclusion that databases for the entire CR are necessary for obtaining the maximum information.

Haplotype distribution of eight Y chromosome STRs in a Southwestern Spanish population sample

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Haplotype distribution, allele frequencies and diversity values of eight Y chromosome STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385I/II -minimal haplotype-) were determined for a population sample of 217 individuals from Andalusia and Extremadura (Southwestern Spain). The data will be sent to the YHRD – Y Chromosome Haplotype Reference Database.

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Y Chromosome Polymorphisms in Argentine Population

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Short tandem repeats (STRs) loci are the most informative PCR based genetic markers available to date for attempting to individualize biological material. The full use of DNA typing technology in forensic science has grown up by the development of National DNA databases. That is the reason why today, many efforts are made to build up Y STRs databases for forensic purposes. Knowledge about mutation rates and mutational process of short tandem repeats (STRs), microsatellite loci used in paternity testing and forensic analysis, is crucial for the correct interpretation of genetic profiles.
In our study, we analyzed Y Chromosome Polymorphisms for the loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS439, DYS438, in unrelated Argentine individuals, most of them from Buenos Aires. Statistical interpretation of the results let us create a database of our own population, and we also studied paternity cases to discover genetic inconsistencies in father-son biological relationship testing.

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Sequence Polymorphisms of Mitochondrial Control Region DNA in Argentine population

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As mitochondrial analysis offers great potentials for individualization in forensics, it is sometimes the more convenient and more successful method to be considered, in particular, with low DNA samples. That is the reason why today, many efforts are made to build up mitochondrial databases for forensic purposes. In our study, we analyzed the Sequence Polymorphisms of Mitochondrial DNA Control Regions, HV1 and HV2, in Argentine individuals, most of them from the city of Buenos Aires, with the aim of creating our own database.

Sequencing of Mitochondrial DNA D-Loop Region [1, 2] has been incorporated since 1993 in our laboratory to study maternal lineage. Since then, it has become a tool of choice for forensic casework. Automated DNA Sequencing of PCR products have made mitochondrial DNA analysis easier and faster. The aim of this study is to analyze Sequence Polymorphisms of Mitochondrial Control Region DNA [3] in our population, and for that purpose, we studied the D-Loop Hypervariable Regions 1 and 2 (HV1 and HV2). Statistical interpretation of the results let us create a database with sequences of our own population.

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In-house validation, casework application and Swiss population data with a new commercial Y-chromosome specific STR multiplex

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The new commercial Y-STR multiplex PowerPlex®Y includes the 11 Y-chromosome STR loci DYS19, DYS385a/b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS 393, DYS437, DYS438 and DYS439.

In-house validation
The PowerPlex®Y multiplex was found to have a detection limit of 0.1ng of template DNA, to give a full male profile in the presence of excess female DNA (ratio 1:50), and to be able to identify the minor component in a male/male mixture down to the ratio 1:5. In average Stutter peaks were $< 15\%$ compared to the main peaks for all loci.

Casework application
52 mixed samples with a female main profile were analyzed. A male profile (at least 9 of the 11 loci) was obtained for 36 (69%) of the 52 samples, including stains from a variety of different materials. The multiplex also worked well for paternity cases.

Swiss population data
Allele frequencies and haplotypes for the 11 Y-chromosome STR loci were determined for a Swiss population sample of 150 unrelated male individuals. 141 different haplotypes were identified (haplotype diversity: 0.9922). The gene diversities for the different loci were in the same range as shown for other European Population samples.

Validation of a Y chromosome specific, 17-locus fluorescent STR multiplex

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Current interpretation of sample mixtures that are analyzed with autosomal markers via PCR can suffer from competition between a relatively small amount of male DNA and a large amount of female DNA. These specimens require the forensic analyst to make a judgment call on which autosomal STR loci correspond to the major and minor contributor. A multiplex PCR amplification system targeting Y-specific loci yield a high degree of confidence that only the male contributor is being analyzed. We are currently in development and validation of a 17-plex Y-STR system with an increased discriminatory capacity over current commercial Y-STR assays. The Y-STR multiplex includes all the loci in the "European minimal haplotype"; DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and the SWGDAM recommended Y-STR loci; DYS438, DYS439. Additionally, we have included six highly polymorphic loci DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4) and Y GATA H4. To ensure no overlap between allele ranges, loci were labeled with 6 - FAM™, VIC™, NED™, and PET™ dyes. We have also generated an extensive allelic ladder containing 137 alleles. The multiplex amplification conditions were optimized on the Gene Amp PCR system 9600 and 9700 thermal cyclers. The amplified products are separated on Applied Biosystems Genetic Analyzer instruments that support G5 matrix analysis such as the ABI PRISM® 3100, 3100-Avant and 310 Genetic Analyzers.

Validation studies demonstrating the effectiveness of the Y-STR multiplex will be presented including male to female, male to male, sensitivity and species specificity studies.

The Y-STR PCR amplification system described, used in conjunction with the Quantifiler™ Y Human Male DNA Quantification Kit, produce reliable and accurate Y haplotypes from the types of samples seen in the forensic laboratory. The system can be used in conjunction with a number of Applied Biosystems Thermal Cyclers and ABI PRISM® Genetic Analysers, and provides the forensic scientist with a complete set of tools for Y chromosome analysis.

Y-STR analysis of Sub-Saharan African populations

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We present the frequency distributions of 11 Y-specific STR polymorphisms (DYS19, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393 and YCAII, i.e., the extended haplotype format of the Y-STR Haplotype Reference Database, YHRD) and the frequency of the combination of these haplotypes in African males.

DNA, that had been extracted from 476 African males originating from West African (e.g., Nigeria, Ivory Coast) to East African countries (e.g., Kenya, Tanzania), served as template to amplify the loci of the YHRD extended haplotype by means of different multiplex or singleplex approaches. PCR fragments were analyzed using ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and sequenced allelic ladders.

In 476 African samples, 348 different haplotypes were observed. Of them, 286 haplotypes were unique and the others were shared by 2 or 7 persons.

A YHRD search (without YCAII) revealed several matches to African samples from the database and only a few to other Non-African samples. These samples will be further investigated, e.g., by Y-SNP typing we will determine the haplogroup to exclude the possibility that European Y-chromosomes had been sampled in Africa.

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Phylogenetic studies of Swedish males by Pyrosequencing of Y-chromosome SNP markers

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Analysis of Y-chromosomal biallelic markers has become important because of their usefulness in forensics and archaeology. Therefore we have, together with our previous study, designed and established a Pyrosequencing (Biotage, Sweden) methodology for studying 17 biallelic Y-chromosome markers (SRY1532, YAP, SRY4064, M35, M78, M89, M201, M170, M26, M223, M253, 12f2, M9, Tat, 92R7, M17 and M269). PCR was designed to produce amplicons around 100 bp, suitable for analysis of highly degraded DNA.
We also present a population study on 305 Swedish males living in seven different regions. Genomic DNA from the males was typed for the 17 markers mentioned above. The population could thus be divided into 16 different haplogroups. Together with results from minimal haplotype Y-STR-analysis population parameters will be calculated, for example FST statistics, diversities and others. According to preliminary data, Västerbotten significantly differs from other regions which is also shown by Roewer et al (*Hum Genet* in press).

STR-haplotype diversity of the Y-chromosome in three Eastern Europe populations

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Polymorphism of Y chromosome microsatellite loci are very useful tool to investigate the origin and the history of human population. Seven Y-chromosome-specific human STR-loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393) were analysed in 403 males with Slavic origin from three Eastern Europe populations – Belorussia, Russia and Ukraine. The allelic variation analysis has not revealed differences between these populations, but highly significant differences were observed when comparing the populations under investigation with population from West Europe. To demonstrate affinity between Belorussian, Russian, Ukrainian and other populations Neighbour-Joining tree based on Nei distances was constructed.

Seven Y-chromosome STR-loci were found to generate 140 different haplotypes in three Eastern Europe populations. Assuming a stepwise mutation model the median-Joining microsatellite network was constructed for most common haplotypes. It is shown that the several ancestral neighbour's haplotypes taken part in formation of people from these three populations on a male line.

The most common haplotypes for three Eastern Europe populations were respectively 16 / 13-32 / 24 / 11 / 11 / 13, 16 / 13-29 / 25 / 10 / 11 / 13 and 16 / 13-30 / 25 / 11 / 11 / 13 for Belorussian, Russian and Ukrainian populations. Comparative analysis of the most common observed haplotypes among worldwide populations using YHRD was performed.

Assessing the suitability of STR loci selected from Y-chromosomal sequence data for forensic use in South Africa.

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Population data for the loci of the "minimal haplotype" have been reported for South African Xhosa and Caucasian communities. The objective of the present study was to identify additional Y-STR loci suitable for forensic casework in these populations. Available Y-chromosomal sequence was surveyed for STR sequences. This lead to the identification of 232 trinucleotide, 437 tetranucleotide and 118 pentanucleotide STR sequences. While many of these loci had already been already been submitted to the Genome Database (GDB) little data was available on their suitability for forensic casework. Sequences were ranked according to the degree of homology between repeated units and the number of repeat units present. Unlabeled PCR primers were designed for twenty five loci. Male specificity, copy number and polymorphism were assessed for 46 Caucasians using polyacrylamide gel electrophoresis and silver staining. Thirteen loci were selected for further analysis using dye-labeled PCR primers combined into two multiplex PCR reactions. Over 100 Caucasian and 75 Xhosa samples were typed. Gene diversity, the number of alleles identified and the average stutter was determined for each locus.

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SNPs of the coding region of mtDNA – first experiences

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Although STR typing is the most useful in human identity testing, the success depends on quality and quantity of genomic DNA extracted from the evidence specimens. Sequence analysis of mitochondrial DNA control region is frequently the only option, when amplification of autosomal DNA is failed. But there are additional markers located in the coding region of mtDNA, which can also be used in forensic practice. Brandstätter et al. (2004) presented a set of 16 SNPs of the coding region of the mtDNA which define haplogroups. First results of testing different populations were presented at the Annual Meeting of the German Society of Legal Medicine. The observed power of discrimination in the tested population was ~ 0.9 and the random matching probability $\sim 10\%$. This study presents analysis of these SNPs in few authentic forensic cases. Sensitivity and selectivity tests were performed. The detection limit for haplogroup typing was a DNA concentration of 0.5pg. DNA-mixtures could be identified up to a proportion of 1:10. These results show the general usefulness of this method analysing special samples in forensic routine case work.

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Y-SNP typing by SNaPshot in the Salvadorean population (Central America).

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Analysis of single nucleotide polymorphism markers on the Y chromosome (Y-SNPs) could provide useful information in medical diagnostics, population genetics and identity testing.
Single nucleotide polymorphisms have been estimated to occur in 1 out of 1000 nucleotides in humans, and therefore embody the most abundant source of genetic variation so far available.
16 Y-SNPs markers examined defined haplogroups of interest in the Salvadorean population using the SNaPshot technique. SNPs examined in this study were M22, P25, 92R7, SRY-1532, M173, M70, M9, Tat, M213, M170, M62, M172, M26, M201, M3, 1272.
SNPs were divided in two multiplex and two singleplex PCRs. SNaPshot products were run on ABI 3100 and analysed using Genescan 3.7 software (Applied Biosystems).

The extent of human mtDNA contamination in bones and teeth from dogs calls for extreme precaution in ancient DNA studies

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DNA investigated in forensic casework and archaeological studies is generally highly degraded and present only in small amounts. As a consequence, molecular damage and contamination with exogenous DNA, renders difficulties in the interpretation of obtained DNA sequences. Authentication of results is especially difficult when working with human samples due to the frequent occurrence of modern human DNA contamination in aDNA extracts. Although this problem is not fully resolved, few efforts have been made to identify the nature of contamination. , Here we investigated the extent and origin of human DNA contamination in one of the most frequently used sources for aDNA studies, that is, bones and teeth from museum collections. In order tTo distinguish contaminant DNA from authentic DNA we extracted DNA from 29 Neolithic, Medieval and modern dog specimens (Canis familiaris). We monitored the presence of a 148 bp human specific and a 152 bp dog specific mitochondrial DNA (mtDNA) fragment in DNA extracts as well as in negative controls. The total number of human and dog template molecules were quantified using real-time PCR and the generated PCR products were sequenced. We found that all dog samples contained contamination from human DNA molecules, often at levels exceeding the amount of authentic ancient dog DNA. We also found that the contaminating human DNA originated mainly from the bones and teeth rather than from laboratory procedures. This leads us to believe that the commonly applied procedures for decontamination of specimens do not provide clean enough samples. Thus, greater efforts are needed to develop more efficient cleaning methods in order to ascertain reliable and reproducible authentic aDNA results.

Y-chromosome polymorphism data in two distinct ethnic groups residing in northeastern Poland

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Population: Ancestors of contemporary Polish Tatars arrived to Poland in the 14th century from the Golden Horde and Khanates of Kazan, Crimea, Volga and Astrakhan. Tatars who presently live in Poland are Sunni Muslims. They number about 2,500 and all speak Polish. Islamic centre of Polish Tatars is Bialystok in the region of Podlasie (NE Poland) where an intensive settlement of Tatars in late 17th and early 18th century took place. Despite several centuries of cultural and religious assimilation and separation from Tatar-Turkic roots Polish Tatars living in separate, closed communities, preserved their ethnic and religious identity. Old Believers are a fraction of the Russian Orthodox Church who came into existence as a result of schism introduced in 1653-1666 by Patriarch Nikon in opposition to the Russian Church Reform, adopting the liturgy and practices of the Greek Church. The Old Believers were severely persecuted under the tsars and sought shelter in the most remote corners of Russian Siberia as well as abroad, including USA, Lithuania and Poland. Many communities lived in almost complete isolation for centuries. Presently, not many more than 600 Old-Believers inhabit Suwalki Region in northeastern Poland, where they founded several villages and have struggled to maintain their religious identity and traditional ways of life. Buccal swabs were collected from unrelated healthy Polish Tatars and Old Believers ($n = 124$, respectively).
Extraction and PCR: DNA was extracted using the Chelex 100 and proteinase K protocol. The quantity of recovered DNA was determined spectrophotometrically.0.5-1ng target DNA was amplified in PCR System 9700 (Applera) using commercial kits: PowerPlex Y System (Promega) or genRES DYSplex-1 and genRES DYSplex-2 (Serac). The SWGDAM recommended Y-STR core set of minimal haplotype was considered (DYS391, DYS390, DYS389I/II, DYS385, DYS392, DYS393, DYS19).
Typing: Electrophoresis and typing were performed in the ABI 310 Genetic Analyzer (Applera). Reference ladders included in the kits were used for genotype classification. The nomenclature according to the Y-STR Haplotype Reference Database (`http://www.yhrd.org`) was used.
Analysis of data: Allele frequencies for each locus were calculated by simple gene counting method. Gene or haplotype diversity/discrimination indices values were calculated as $\mathrm{GD} = 1 - \sum_i {p_i}^2$, where p is the frequency of the i-th haplotype or allele. Haplotype discrimination capacity was calculated as $\mathrm{DC} = \frac{H}{N}$, where H is the total number of different haplotypes and N is the total number of individuals in the sample. Analysis of molecular variance (AMOVA) was performed using the Monte-Carlo test included in the Arlequin software ver. 2.000.
Results: The combined values of GD were 0.9638 and 0.9938 for Polish Tatars and Old Believers, respectively with corresponding values of $\mathrm{DC} = 0.81$ and 0.79, respectively. The pairwise population comparisons based on AMOVA estimates revealed significant differences between the autochthonous Poles and Polish Tatars ($R_{ST} = 0.0127$, $p = 0.0090$) and between the autochthonous Poles and Old Believers ($R_{ST} = 0.0311$, $p = 0.0000$). These data support the idea of a Polish admixture in Y-chromosomal lineages of Polish Tatars. Old Believers appeared to be more distant from autochthonous Poles which may reflect their different history, religious affiliation and long-established principles of living.

Y-chromosome polymorphism data in three major population groups in northeastern Poland

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Population: Ethnically, Poland has a largely homogeneous population, its percentage of national or ethnic minorities being one of the lowest in Europe, officially estimated at between 3-4% of the population, which is equivalent to about 1.5 million people. Podlasie in northeastern part of Poland is a frontier region where the influences of various countries and cultures have been clashing for centuries. The region differs from the others due to its scanty population (1.2 million) and ethnical and cultural diversification. In nationwide census, which was carried in the year 2002, Polish citizenship was declared by 46,041 persons of Belarussian nationality and 5,639 persons declared Lithuanian citizenship. It is estimated unofficially however, that northeastern corner of Poland is inhabited by 200,000-300,000 Belarussians and 20,000-30,000 Lithuanians. Lithuanians compose one of the most emancipated, best organized and least assimilated minority communities in the country, the linguistic factor playing a crucial role in maintaining their regional and national identity. The majority of Polish Lithuanians are Roman Catholic Christians, while most Belarussians are followers of the Polish Autocephalous Orthodox Church. Buccal swabs were collected from unrelated healthy autochthonous Polish males and individuals of the Belarussian and Lithuanian minorities ($n = 124$, respectively).
Extraction and PCR: DNA was extracted using the Chelex 100 and proteinase K protocol. The quantity of recovered DNA was determined spectrophotometrically.0.5-1ng target DNA was amplified in PCR System 9700 (Applera) using commercial kits: PowerPlex Y System (Promega) or genRES DYSplex-1 and genRES DYSplex-2 (Serac). The SWGDAM recommended Y-STR core set of minimal haplotype was considered (DYS391, DYS390, DYS389I/II, DYS385, DYS392, DYS393, DYS19).
Typing: Electrophoresis and typing were performed in the ABI 310 Genetic Analyzer (Applera). Reference ladders included in the kits were used for genotype classification. The nomenclature according to the Y-STR Haplotype Reference Database (`http://www.yhrd.org`) was used.
Analysis of data: Allele frequencies for each locus were calculated by simple gene counting method. Gene or haplotype diversity/discrimination indices values were calculated as $\mathrm{GD} = 1 - \sum_i {p_i}^2$, where p_i is the frequency of the ith haplotype or allele. Haplotype discrimination capacity was calculated as $\mathrm{DC} = \frac{H}{N}$, where H is the total number of different haplotypes and N is the total number of individuals in the sample. Analysis of molecular variance (AMOVA) was performed using the Monte-Carlo test included in the Arlequin software ver. 2.000.
Results and conclusions: The combined values of GD were 0.9836, 0.9750 and 0.9815 for Poles, Belarussian minority and Lithuanian minority, respectively with corresponding values of DC = 0.84, 0.86 and 0.82, respectively. The pairwise population comparisons between autochthonous Poles the Belarussian minority and between autochthonous Poles the Lithuanian minority revealed statistically significant differences ($p = 0.0180$ and 0.0360, respectively). Furthermore, relatively small values of interpopulation variation ($R_{ST} = 0.0064$ and 0.0162, respectively) indicate a certain degree of genetic differentiation between the autochthonous population and the both minorities. The resulting data are consistent with the idea of a genetic proximity to the Polish population due to the common Slavic origin and historical-political contacts. We suggest that the differences in some haplotype frequencies should be taken into consideration in certain trace-donor match analyses within the population of northeastern Poland.

The detection of microdeletions in MSY region with STS and STR loci

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In 40% of the childless families infertility of the male stands behind, in 37 % of these cases the problem appears to be genetic. The diagnostic tools that could test the genetic differences between the healthy and azoospermic males are not very efficient. The genes that are responsible for the production of the azoospermia factor (AZF) are located on the long arm of the Y chromosome in the male specific region (MSY). The deletion of AZF region is thought to be pathogenetically involved in some cases of male infertility associated with azoospermia or severe oligozoospermia. The (micro)deletions on the long arm of Y chromosome (Yq) define three regions (AZFa, AZFb, and AZFc) associated with failure of spermatogenesis. The Y chromosome specific sequence-tagged site (STS) and short tandem repeat (STR) markers are capable of identifying microdeletions in samples from peripheral blood lymphocytes of infertile males. Until now all STR markers that are commercially used in forensic genetics are located in the AZFa and AZFb regions. In 40 samples from males with azoospermia both the STS and STR detection were performed. In 2 samples the deletion affected both the AZFb and AZFc regions and in another 2 samples partial deletion of AZFa appeared to be the cause of azoospermia. Supposedly, in 2 samples the complete translocation of the Yp with loss of Yq occurred since only the markers on the Yp could be detected with both methods. Phenotypically both of them are males with 46, XX karyotypes. The DYS464 marker is located in the AZFc region very near to the DAZ (Deleted in Azoospermia) genes. The DYS464 marker appears to be the most polymorphic Y-STR marker discovered to date. A single primer pair can generate up to eight copies, which on the ABI 310 Prism Genetic Analyzer might show up to four distinct peaks. The peaks can reveal double, triple or more copies. Allele calls can be made based on peaks that are present and peak height ratios. There are four DAZ genes and they are important for spermatogenesis. The application of the DYS464 marker for detecting microdeletions is now in experimental stage and requires the balanced quantification of the PCR products with an internal standard. The DYS447 marker is a suitable marker as standard, since it is located between the AZFb and AZFc region and the sizes of the PCR products - the detection of the marker was developed before in the laboratory of John Butler (2003) - are very similar to that of the DYS464. Eighty Hungarian male samples were tested for DYS464 for allele sizing and the detection of the copy numbers in the healthy Hungarian population. Samples from infertile males with azoospermia are in progress for testing microdeletions and so far in three samples the deletions in AZFc region with both STS and DYS464 detection method were confirmed. The development of the duplex PCR reaction raises the question of which DAZ gene(s) is affected by microdeletion(s). Further PCR markers adjacent to the DAZ genes are yet to be developed.

Y-chromosomal STR haplotypes in Sicily

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The reconstruction of the genetic history of Sicily is extremely difficult, because of the complexity of human movements and settlements that interested this area of the Mediterranean Sea in prehistorical and historical times. Previous studies, based on classical and DNA markers (autosomal STRs and mtDNA), are discordant regarding the presence of genetic heterogeneity within the island, some of them suggesting a geographical pattern of differentiation following a longitudinal (East- West) axis. Analysis of Y-chromosome biallelic polymorphisms showed a haplogroup frequency distribution similar to Southern Italy and Greece. In this study, Y-STRs currently used to define the minimal haplotype employed in the "Y-STR haplotype reference database" (DYS19, DYS389I-II, DYS390, DYS391, DYS392, DYS393, and DYS385) were typed in 215 unrelated males from Sicily. Individuals represented in the population sample were chosen from both geographically and historically distinct areas of the island: Trapani, Santa Ninfa, Alcamo (Western Sicily); Sciacca, Caccamo, Mazzara del Vallo (Central Sicily); Troina, Ragusa, Piazza Armerina (Eastern Sicily). Comparison of haplotype distributions was carried out between different sampling regions and with neighbouring Mediterranean populations.

The obtained results, combined with information from Y biallelic markers, will hopefully contribute to the understanding of the genetic landscape of the island. Furthermore, a geographically detailed reference database of Sicilian Y-STR minimal haplotypes can be of practical help to forensic scientists performing paternity testing and human identification.

Evaluation of 52 novel Y-STRs for forensic and population-genetic studies

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A recent comprehensive survey of Y-STRs (Kayser et al. 2004, *Am. J. Hum. Genet.* **74**, 1183-1197) identified 139 new polymorphic loci. Fifty-two of these were present in a single copy and had simple structures, and are being evaluated further. We have assembled them into 16 small multiplexes using the AutoDimer software (Vallone and Butler 2004, *BioTechniques* **37**, 226-231) to avoid primer-dimer interactions, and optimised the amplification conditions, including magnesium concentration, annealing temperature, and primer concentration. The final conditions use touchdown PCR with differently labelled primers, and the products are analyzed on an ABI3100 genetic analyzer. The 76 samples of the Y Chromosome Consortium (YCC) panel are currently being typed. Some markers, for example DYS481 and DYS570, are highly polymorphic showing up to 11 alleles, while others, such as DYS472 and DYS530, are less polymorphic and show only two alleles. Additional diverse populations will be typed in the future. We hope that these markers will contribute to both forensic investigations and human evolutionary studies.

Y-chromosomal STR haplotypes in Macedonian population samples

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Eleven Y-chromosomal short tandem repeats (STRs), DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438, DYS439 were typed in DNA samples from Macedonian population ($n = 150$). The Macedonian population sample has two dominant minimal haplotypes with frequencies $> 5\%$ (13,13,30,24,10,11,13,16-18 and 16,13,31,24,11,11,13,14-15). By haplotype extension (DYS437, DYS438, DYS439) both of these minimal haplotypes can be further split up into 4 different, 1-allele step deviant chromosomes, respectively. The frequencies of the 20 most frequent minimal haplotypes in the Macedonian population have been compared with those from populations inhabiting 4 neighbouring geographic regions sampled for the YHRD, i.e. Albania ($n = 101$), Bulgaria ($n = 122$), Croatia ($n = 150$) and Greece ($n = 101$). The analysis revealed the most pronounced similarities in the minimal haplotype frequency with the Croatian and Albanian samples. The gene diversity for the minimal haplotype was 0.9838, for the 11-locus haplotype 0.9889. One haplotype analysed in this study includes a duplication of the locus DYS19 (alleles 15,16), a type of mutation which has been observed so far with a frequency of about 0.05% in $21,546$ individuals in the YHRD (release 10 from February 26, 2004).

D-Loop variability in Japanese, Vietnamese and in Peruvian Amerindians sharing the intergenic CoII/tRNA^{LYS} 9bp deletion

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Using a simple PCR/PAGE test we screened samples of six populations to detect the 9bp deletion which is located in the intergenic CoII/tRNA^{LYS}. Frequencies were estimated as follows: Amerindians from Peru: 32/73 (43%), Vietnamese: 30/120 (25%), Japanese14/137 (10%), Bantu from Cameroon 1/50 (2%), Germans: 0/350, Sami from Norway 0/25. Individuals from the different populations sharing the 9bp deletion were compared in regard to their HV1 and HV2 sequences.

Genetic diversity of three Singapore populations using Y-Short Tandem Repeat and mitochondrial D-loop loci

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Human chromosome Y-specific Short Tandem Repeats (Y-STRs) loci and mitochondrial DNA (mtDNA) D-loop sequence are useful markers in forensic analysis, genealogical and evolutionary studies. This study has established two population databases of Y-STR and mtDNA D-loop haplotypes for the three main ethnic groups in Singapore, namely Chinese, Malay and Indian.

For the Y-STR database, 11 Y-STR loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438 and DYS439) were genotyped for 210 Chinese, 186 Malay and 183 Indian, all unrelated individuals with self-reported ethnicity. Locus diversity ranged from 0.359 to 0.965. A total of 534 haplotypes were found in all three ethnic groups, consisting of 191 haplotypes in Chinese, 175 in Malay and 174 in Indian, with 6 haplotypes shared between 2 populations. Unique haplotype in Chinese, Malay & Indian numbered 178, 165 and 167 respectively. Haplotype diversity of the 11 Y-STRs was calculated to be 0.9987, 0.9993 and 0.9993, while discrimination capacity 90.95%, 94.09% and 95.08% for Chinese, Malay and Indian respectively.

For the mtDNA database, the 1.2kb control (D-loop) region containing the two hypervariable (HV1 & 2) regions and other variable sequences commonly used in population and forensic genetics were sequenced in 99 Chinese, 70 Malay and 95 Indian individuals. The observed frequency of a random match between pairwise sequences was 0.36% (HV1 only), 1.2% (HV2 only), 0.15% (HV1+2) and 0.13% (whole CR). Additional CR sequence outside HV1 and HV2 contributed marginally to discriminativeness. Computed random match probability (RMP) and haplotype diversity for HV1+2 were 0.011, 0.018, 0.012 and 0.9990, 0.9967, 0.9989 for Chinese, Malay and Indian respectively. The mean sequence differences for HV1+2 were 11.43 (Chinese), 11.26 (Malay) and 10.47 (Indian), and were comparable to other defined populations (7.2-14.1).

Genetic distances comparison between populations using both Y-STR and mtDNA markers indicated Chinese-Malay are the most related, while Chinese-Indian are the furthest apart genetically. To our knowledge, this study represents the most comprehensive report of Y-STR and mtDNA haplotype distribution in Singapore to date. Analysis of the data provided a glimpse of the genetic diversity of the three Singapore populations. Comparing the patterns of Y- STR and mtDNA variation provided some insight into the paternal and maternal history of the three populations.

Y-SNPs – current problems with introducing a new tool for forensic purposes

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Our genome could be considered a mosaic distinctive of each individual. This uniqueness is related to several sources of genetic variation which include single nucleotide polymorphisms (SNPs). A large number of SNPs located in the male specific portion of the Y- chromosome has been established and characterize most of the Y-chromosome variability present in Europe. Even if these SNPs provide limited information in routine case- work analysis than STRs they could be useful for particular male-identification cases.
Based on our experience we accessed the current problems concerning Y- chromsome-SNP typing and the related validation strategies.
A rare polymorphic site defined by the M18 mutation has been analyzed with two different genotyping methods and will be used as an example to discuss advantages and limitations of the various typing approaches. The utility and drawbacks of high-throughput platforms in forensic practice will be underlined. Particular emphasis will be placed on the need of generating short amplicons for the informative SNPs defining the main Y-chromosome European lineages.

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