O1

Application of Y-STR's in forensic routine cases

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The Y-STR's are established for forensic routine cases at the last years. Especially in cases of deficiency, identification and rape cases these markers are powerful. Large databases were established for free online-using to calculate the frequencies of the detected haplotypes - www.ystr.org - in different populations. We use the so called minimal core set of Y-STR's in all paternity routine cases and also in rape cases or cases of sexual abuse. To solve the throughput problems we tested several multiplex reactions. Finally we established in addition to the Y-Plex-Kit of Reliagene a duplex-PCR DYS389/DYS392. The PCR products can be pooled and only one run on the ABI310Prism Genetic Analyser is necessary. The results show the high sensitivity of the reactions. Several examples of our routine casework will be presented. In a part of this cases the autosomal STR's which are used for the German national database of criminal offenders show no mixture of male and female stains. The analysis of Y-STR's gave the possibility to solve the cases and convict the suspects. In such cases we suggest to analyse Y-STR's first to prevent any unnecessary loss of target DNA. In our hands the highly concentrated female DNA does not interfere with the detection of the male DNA. Costly and time-consuming manipulations like differential lysis or micromanipulations are not necessary. The biostatistical calculations (either haplotype counting in different populations, frequency estimation, or calculation of discrimination indices) can be done simply by using the YHRD website for the continent concerned (USA, Europe, Asia).

O2

Persisting fetal microchimerism is not a problem in forensic Y-STR typing

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At the second Y-User Workshop we highlighted the concept of persisting fetal microchimerism, the phenomenon of engraftment of fetal (stem)cells into maternal tissue during pregnancy. These cells can persist for decades in various tissues of the maternal body. We speculated that this phenomenon might interfere with forensic Y-STR typing, as the engrafted cells of mothers of sons possess a Y-chromosome. Various immunological researchers have confirmed microchimerism in the last years. However, the question wether these obviously minute "internal contamination" is actually capable of producing false positive results in forensic Y-STR typing still remained unanswered, especially as the studies on microchimerism were performed using a Y or HLA specific nested PCR with 45 to 60 cycles, whereas forensic scientists usually restrict the number of PCR cycles to 30. We have thus tested a Y chromosomal marker (SRY) in DNA extracted from the blood of 66 mothers having given birth to at least one son 6 to 18 months before phlebotomy and in DNA extracted from vaginal swabs of 9 mothers of at least one son who have had no sexual intercourse in the last 2 weeks before sampling. We were not able to detct a Y-chromosome specific product in any of the samples when using 10 ng of DNA and 30 PCR cycles. However, when increasing the quantity of DNA to 200ng and the number of PCR cycles to 45, 6 of 9 vaginal swabs and 14 of 66 blood samples were positive for SRY. This study shows that persisting fetal microchimerism is not capable of generating false positive results in forensic Y-STR typing when obeying basic rules for good laboratory practice. It also emphasizes that extending PCR to substantially more than 30 cycles should not be an option in forensic DNA profiling.

O3

Multiplex PCR development of Y-chromosomal biallelic polymorphisms for forensic applications

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The microsatellites of the Y-chromosome are useful in forensic casework analysis involving male subjects, such as paternity testing, identification of bloodstains or other biological evidence with mixed profiles and reconstruction of family relationship by patrilineage analysis. More recently, there is an increasing development of biallelic polymorphism (SNPs) associated to the nonrecombining portion of the Y-chromosome defined by a single mutation or, rarely, by deletion or insertion di one or few nucleotides. Low mutation rate, paternal heritage only and absence of recombination, make them particularly suitable for evolutionary studies.

Moreover, such biallelic polymorphisms are of special interest for forensic purposes, because the amplification of short fragments including the single base mutation which characterizes their polymorphism potentially allows a positive result even with high-degraded DNA, where microsatellites fail. Since several loci have to be examined to achieve a sufficient degree of informativeness, requested in forensic caseworks, multiplex PCR from minimal amounts of DNA have to be developed.

The aim of this study is to set-up multiplex PCR of NRY SNPs suitable for forensic purposes. A first multiplex has been developed with SNP loci defining the European haplogroups (M35, M89, M172, M170, M9, M173, M45). PCR was performed with primers designed to produce amplicons in a range between 96 and 136 bp starting from 1 nanogram of DNA template. PCR product was minisequenced with tailed primers of different length and run in an automated 5-colour capillary electrophoresis sequencer.

O4

Development and Characterization of Y Chromosome Markers for Forensic Casework

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We have attempted to improve the discriminatory potential, and hence the probative value, of Y-STR based testing by extending the set of Y chromosome STR loci available for forensic use. In accord with the requirements of a Y chromosome multiplex analytical system developed specifically for forensic use, we have sought to maximize the number of loci able to be co-amplified, ensure appropriate assay sensitivity (1-2 ng of input genomic DNA), balance inter-locus signals and minimize confounding female DNA artifacts.

Two Y chromosome STR systems, multiplex I (MP I) and multiplex II (MP II), have been developed which permit the robust co-amplification of 19 Y STRs and subsequent separation and detection using a standard capillary electrophoresis analytical platform. The loci include DYS19, DYS385(a) and (b), DYS388, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS425, DYS434, DYS437, DYS438, DYS439, Y-GATA-C4, Y-GATA-A7.1, Y-GATA-A7.2, Y-GATA-H4. The two multiplex systems are robust over a wide range of primer, magnesium and DNA polymerase concentrations and perform well under a variety of cycling conditions. Complete male haplotypes can be obtained with as little as 100-250 pg of template DNA. Although a limited number of female DNA artifacts are observed in mixed stains in which the male DNA comprises 1/100th of the total, the male profile is easily discernible. Slightly modified versions of MPI and MPII demonstrate a significant reduction in female artifacts. Thus, it may not be necessary to employ a differential extraction strategy to obtain a male haplotype (or haplotypes in the case of multiple male donors) in cases of sexual assault. The potential utility of MPI and II for forensic casework is exemplified by their ability to dissect out the male haplotype in post-coital vaginal swabs taken several days after intercourse.

Two additional Y chromosome STR systems, Multiplex III and Multiplex IV, are under development that should, in combination with the MPI/II systems, extend the number of loci available for forensic use to at least 48.

This study has emphasized the need for novel Y-STR multiplexes developed for forensic use to undergo a series of validation exercises that go beyond simply optimizing the PCR reaction conditions. Specifically, stringent performance checks on their efficacy need to be carried out using casework type specimens in order to determine potential confounding effects from female DNA.

O5

A Y-Chromosome SNP multiplex for forensic genotyping

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We have developed a robust Single Nucleotide Polymorphism (SNPs) typing assay with co-amplification of 25 DNA-fragments and the detection of 35 SNPs on the Y-chromosome. Y chromosome fragments of 80 – 180 bps with known SNPs were selected. Amplification primers with theoretical melting temperatures about 60°C were designed. The concentrations of amplification primers were adjusted to obtain balanced amounts of PCR products in high concentrations of $MgCl_2$. For routine purposes, 10 ng of genomic DNA was amplified. A detection primer was designed for each SNP. In order to distinguish between the sizes of the 35 detection primers, the detection primers were synthesized with different 5'-poly-C tails. The lenghts of the detection primers ranged from 18 to 90 nucleotides at size intervals of 4 nucleotides. Single-nucleotide extension reactions of the 35 SNPs were carried out simultaneously with fluorescently labeled dideoxynucleotides using the ABI PRISM® SNaPshot™ Multiplex kit (Applied Biosystems). The sizes and the coulours of of the extended products were determined in an ABI PRISM 3100 capillary sequencer. The frequencies and haplotype relationships of the 35 SNPs were determined in 200 Danes and 200 persons from the Canary Islands.

O6

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.ystr.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.

O7

Y-Chromosome STR Genotyping for 11 Loci: Development and Validation of the Y-PLEX™5 and Y-PLEX™6 Systems for Forensic Casework

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The polymorphic short tandem repeat (STR) loci residing on the non-recombining region of the Y-chromosome have become increasingly important in forensic DNA analysis. This is because it is possible to type the male DNA specifically in a sample containing mixtures of male and female DNA.

We have developed two genotyping systems: Y-PLEX™5 for amplification of the DYS389I, DYS389II, DYS392, DYS438 and DYS439 loci and Y-PLEX™6 for amplification of the DYS19, DYS385, DYS389II, DYS390, DYS391 and DYS393 loci. Analysis of samples using Y-PLEX™5 and Y-PLEX™6 provides results for all nine minimal haplotypes and two additional markers DYS438 and DYS439. Allelic ladders were generated for each genotyping system. Alleles in the allelic ladders were sequenced to confirm the allele number which were further verified by sample exchange with the Institute of Legal Medicine, Humboldt-University Berlin, Germany, Institute for Pathology and Molecular Immunology, University of Porto (IPATIMUP), Portugal and National Institute of Standards and Technology (NIST), USA.

The validation studies for these systems included the following experiments: annealing temperature, primer ratio, primer concentration, salts, DNA polymerases, dNTPs, thermal cyclers, denaturation time, annealing time, cycle extension time, final extension time, PCR cycles, reaction volume, female DNA, sensitivity, non-human studies, reproducibility, precision, additives, inter-laboratory studies, female-male mixtures, male-male mixtures, stutter, DNase degradation, environmental insult, and non-probative casework. The results for some forensic cases analyzed using the Y-PLEX™6 system will be discussed. A database for 11 alleles was generated for Caucasian, African American and Hispanic population groups. The haplotype frequency and genetic diversity using these 11 alleles will be presented. The database with a haplotype frequency calculator is freely available for use by forensic community.

O8

Application of a novel, thermostable SSB protein-based multiplex PCR system for analysis of three Y-chromosome STR loci in forensic genetics

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The application of Y-chromosome STR polymorphism analysis includes its use in deficiency paternity testing cases and to discriminate stains in forensic investigation. Apart from technical advantages, the ability to analyse several loci simultaneously by multiplex PCR dramatically improves the discrimination power of the systems. Multiplexing different systems often requires redesign of new primers because of potential primer interactions. In this work, we describe a trial of coamplification of three Y-chromosome STR loci: DYS390, DYS392 and DYS393, which appeared to be impossible in standard conditions due to amplification of an incorrect 36-bp product, caused by primer dimer formation. To eliminate this, a completely novel approach in multiplex PCR was worked out. A step of preincubation of primers with thermostable SSB protein eliminated the incorrect interaction and enabled amplification of the triplex system, using the originally described primers. The new system proves usefulness of SSB protein in multiplexing of polymorphic loci used in forensic genetics. It exhibits relatively high haplotype diversity and in connection with other Y-STR systems constitutes an excellent tool in paternity testing and personal identification.

O9

Use of Locus Specific Brackets in Calibration of Y Chromosome STR Alleles

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Locus specific brackets (LSB) make excellent calibration markers because they are derived directly from a target locus by means of recombinant DNA technology and designed to contain just 1-2 fewer or more repeat units than all common alleles of that locus. Therefore, when LSB are loaded into a sample lane for electrophoresis together with the PCR amplicons from their target loci, they will migrate in register just before and after the target alleles of their unique locus of origin to provide excellent calibration of their fragment lengths. Their calibration accuracy is stabilized against run-to-run variation by an external electrophoresis of LSB together with 2 alleles from each locus as part of each group of sample runs.

In the present work LSB were constructed from *extended templates* of 10 Y-STR loci by joining their unique central repeat regions to 200-300 bp of both 5' and 3' flanking sequence where sufficient extended sequence was known. These extended templates provided binding sites for different primer sets created to amplify the appropriate LSB during multiplex PCR design.

The multiplexed STR loci comprised the European haplotype, which are the 8 best studied Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385), and the highly polymorphic YCAII locus, which together with the other 8 loci forms the extended European haplotype. To this group we added the informative and frequently employed DYS388 locus. Since the YCAII and DYS385 primer pairs each amplify two independently polymorphic STR products from reduplicated sites on the Y chromosome, taken together these 10 loci yield 12 polymorphic STR products in a multiplex PCR reaction. After electrophoresis on the Applied Biosystems 310 instrument and processing by GeneScan, the STR amplicon fragment lengths were called from their migration times and LSB calibration by a software program developed for this purpose. There were no amplification artifacts from PCR carried out 5ng DNA samples from a standard test panel of 9 males. PCR carried out on 5ng of female DNA produced no detectable amplicons.

O10

Analysis of Y chromosome SNP's on the Nanogen Molecular Biology Workstation

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There are two main SNP-typing protocols for the Nanogen Molecular Biology Workstation. In the capture down protocol, biotin-labelled capture oligos are electronically addressed to a specific pad on the NanoChip™ cartridge and, subsequently, the PCR product is directed to that pad electronically and the PCR product is hybridized to the capture. In the amplicon down protocol, biotin-labelled PCR products are electronically addressed directly on to the pad. The SNP is detected in both protocols by reporter oligos specific for either the wildtype or the mutant allele. The two reporters are hybridized to the PCR product, and the sample allele is scored by stringent washes at increasing temperatures. We have analysed 7 Y chromosome SNPs using the capture down protocol and 12 Y chromosome SNPs using the amplicon down protocol. Only one of the 7 SNP's tested by the capture down protocol was successfully typed, whereas all 12 SNPs tested by the amplicon down protocol could be typed. In addition, we have analysed various multiplex PCRs by the amplicon down protocol. With this method, more than one PCR product is addressed to the same pad on the cartridge. Subsequent hybridization and detection of reporter oligos is not a problem. However, special attention to the possibility of reporters cross hybridising to other targets is necessary. Secondary structures in the DNA and possible hybridization between the PCR products in the multiplex will impair the hybridization of reporters to the PCR products. Therefore, when testing a multiplex PCR using the amplicon down protocol, the PCR products must be as short as possible.

O11

Accreditation of Y-STR minimal haplotype markers

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In December 1997 the Department of Forensic Genetics received accreditation for genetic analyses in paternity investigations according to the EN 45 001 standard. At that time our analysis consisted of 4 VNTR markers and 3 "in house" developed autosomal STRs. Prior to this accreditation we had set up requirements for these genetic markers. The requirements were mainly based on the Standards for Parentage Testing Laboratories given by the American Association of Blood Banking (AABB) and on recommendations given by the ISFH. Since then we have received accreditation for more "in house" markers as well as the STR-kits Profiler Plus and COfiler. In the beginning of July 2002 our analysis of DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385 markers received accreditation according to the standard ISO/IEC 17 025. The earlier experience from the accreditation of the "in house" markers was very useful when selecting requirements for the Y-STR minimal haplotype markers. These requirements include family studies, genetic information, population studies, measurement errors and participation in QA-programs. I will present these requirements in comparison to the ISFG-recommendations and also give some insight in the documentation we use.

O12

Results of the GEP-ISFG (The Spanish and Portuguese ISFG Working Group) collaborative study on the Y Chromosome STRs: GATA A7.1, GATA A7.2, GATA A10, GATA C4, GATA H4, DYS437, DYS438 and DYS439.

Sánchez-Diz P, Gusmão L, Benítez-Páez A, García O, García-Poveda E, Geada H, Martin P, Martínez-Jarreta B, Pinheiro F, Raimondi E, Silva de la Fuente SM, Vide MC, Whittle MR, Zarrabeitia MT, Carracedo A, Amorim A

GEP-ISFG

A collaborative exercise was carried out by the Spanish and Portuguese ISFG Working Group (GEP-ISFG) in order to evaluate the performance of two Y chromosome STR tetraplexes, which include the loci GATA A7.2, GATA C4, DYS437 and DYS438 (GEPY I), and GATA A7.1, GATA A10, GATA H4 and DYS439 (GEPY II).

In a first stage, the participating laboratories were asked to type three samples for the above markers, using a specific amplification protocol, made available at the website www.gep-isfg.org. In addition, two control samples with known haplotypes, were provided.

The results obtained by the 13 different participating laboratories were identical, except for two laboratories that failed to type correctly the same two samples for GATA C4.

The groups that reported correct results in all the systems were asked to type a small sized population sample in order to evaluate the informative content of these STRs in different populations. A total of 1021 males and 13 different populations, from Argentina, Brazil, Costa Rica, Macao, Mozambique, Portugal and Spain, were analysed for all the loci included in the present study.

Haplotype and allele frequencies of these 8 Y-STRs were estimated in all samples. The lower haplotype diversity was found in the Lara (Argentina) population (95.44%) and the highest (99.90%) in Macao (China)

Pairwise haplotype analysis showed the relative homogeneity of samples of Iberian origin, in accordance with what was previously found in the European populations for other Y-STR haplotypes (www.ystr.org).

As expected, the four non-Caucasian samples, Macao (Chinese), Mozambique (Africans), Costa Rica (Africans) and Argentina (Lara, Amerindians), show high statistically significant F_{st} values in the pairwise comparisons with all the Caucasian samples.

O13

Haplotype analysis of 13 Y-STRs, YAP,SRY465 and M9 in a Japanese population

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Analysis of Y-chromosomal STRs has become increasingly important because of their usefulness in forensic and population genetics. In addition, analysis of SNPs are effectively used in these fields.

We present the frequency distributions of 13 Y-specific STR polymorphisms, YAP element and two SNPs (DYS19, DXYS156, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, YCAII, YAP, SRY465 and M9) and the frequency of the combination of these haplotypes in Japanese males. Y-STR loci, YAP and two SNPs were analysed 125 unrelated males and 114 different haplotypes were observed. A total of 107 haplotypes of them were found to be unique and the others were shared by two to six persons. The haplotype diversity was 0.9972.

As relatively stable polymorphism, four of the eight possible combination haplotypes of YAP, SRY465 and M9 were identified in a Japanese population.

YAP(+)/M9(C)/SRY(C) were 55, YAP(-)/M9(C)/SRY(C) were 11,YAP(-)/M9(G)/SRY(C) were 24 and YAP(-)/M9(G)/SRY(T) were 35.

As focused in DYS385 system, comparison of four haplotype groups demonstrates different frequency-distribution profile among them.

YAP(-)/M9(G)/SRY(T) group had four dominant allele combinations 10-17,10-18,10-19,10-20 which were not found in other three groups.

The high homology of the group suggests that C to T transition in SRY occurred in recently. [1)]

The most common genotype in YAP(+)/M9(C)/SRY(C) group was 13-17. Only two examples having 13-17 were found in YAP(-)/M9(G)/SRY(C).

There was only a small degree of overlap in the four groups.

In conclusion, Y-STRs typing added by SNPs has lead to an informative anthropological and evolutionary studies.

1)Emiko Naito et al. (2001) A novel dimorphism in the human SRY gene: usefulness in human migration studies Int J Legal Med 114 : 274–277

O14

Comparative analysis of STRs and SNPs on the Y-chromosome in Germans, Chinese and Thais.

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We have typed genomic DNA samples from 95 individuals from Germany, 78 individuals from Bangkok/Thailand and 56 individuals from Chengdu/China for 11 Y chromosomal diallelic polymorphisms and 9 STR systems. For SNP analysis, we have introduced a rapid method using the single base extention technology (minisequencing) in combination with capillary electrophoresis. The products from two single PCRs for SRY-8299 and Tat, two duplex PCRs (SRY2627 together with 92R7 and SRY1532 together with M9) and one triplex PCR (M13 together with M17/M19 and M20) were pooled and used as templates for the commercially available SNaPshot kit from Applied Biosystems.

In addition to these ten SNPs we also tested the Y chromosomal diallelic Alu repeat insertion DYS287 (YAP) by agarose gel electrophoresis as well as the Y-chromosomal STR systems DYS19, DYS389I+II, DYS390, DYS391, DYS392, DYS393 and DYS385 by fluorescent multiplex fragment analysis.

Among the 11 diallelic SNP/Alu systems, only six were found to be polymorphic in the three population samples. From these a total number of seven different haplogroups could be identified in the three populations according to the nomenclature by Jobling and Tyler-Smith (2000). Of these, five haplogroups were present in Germans, five in Thais, and only two in Chinese. These haplogroup trees clearly represent population-specific structures. Haplogroup 26 is represented at a high frequency in the Thai and Chinese populations whereas it is absent in Germans.

The Y-STR data confirm a haplogroup-specific distribution of Y-STR haplotypes. Only a few cases of shared STR-haplotypes between different SNP-haplogroups were detected in each of the three populations studied.

O15

A comparison of Y-chromosomal and autosomal STR data in past and present-day Mongolian populations

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In this study, Y-chromosomal short tandem repeat (STR) data obtained from an ancient population (III B.C.-III A.D.) of the Egyin Gol valley (Mongolia, peri-Baïkal area) were compared with those from two contemporary Mongolian populations: one from neighbouring locations (Egyin Gol valley, Selenge, Kantaï) and one from more distant countries. Genetic analyses were performed most often with the Y-Plex6™ and Y-Plex5™ kits according to the manufacturers' recommendations (ReliaGene Technologies Inc.). Biallelic patterns for DYS19 loci were found in both contemporary Mongolian samples, but not in the ancient one. However, two ancient haplotypes were found in the Egyin Gol modern population suggesting a possible genetic continuity with ancient populations. These data together with those of autosomal STRs could allow to draw the evolution of Mongolian populations.

O16

Haplotype-specific Variation of DYS 385 Genotypes in an Austrian Caucasian Population Sample

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The Y-chromosomal short tandem repeat locus DYS 385 is characterized by a duplicated repeat sequence revealing a two-band pattern in male individuals. Compared to other Y-STRs DYS 385 is by far the most informative marker. For this reason DYS 385 is of significant forensic relevance. 80 different DYS 385 genotypes were found in the European Y-STR Haplotype Reference Database (YHRD). Contrary to this remarkable diversity is the non-uniform distribution of the DYS 385 genotypes within the European population. Based on the data from the YHRD 66% of all male individuals show one of the 8 most abundant genotypes. The most common genotype is 11-14 and has a frequency of 31.4% (n=11,078). In a population study on 231 unrelated males from Tyrol we found nearly the same value (30.7%). Additionally we analyzed a set of Y-chromosomal SNPs and STRs. A significant finding was the strong correlation of DYS 385 11-14 with the derived state of the Y-SNP M9 (G-allele), which defines the clade K according to the nomenclature system suggested by the Y Chromosome Consortium.

O17

Homogeneity and distinctiveness of Polish paternal lineages revealed by Y chromosome microsatellite haplotype analysis.

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Different regional populations from Poland were studied in order to assess the genetic heterogeneity within Poland, investigate the genetic relationships with other European populations and provide a population-specific reference database for anthropological and forensic studies. Nine Y-chromosomal microsatellites were analysed in a total of 919 unrelated males from six regions of Poland and in 1,273 male individuals from nine other European populations. AMOVA revealed that all of the molecular variation in the Polish dataset is due to variation within populations, and no variation was detected among populations of different regions of Poland. However, in the non-Polish European dataset 9.3% ($P<0.0001$) of the total variation was due to differences among populations. Consequently, differences in RST-values between all possible pairs of Polish populations were not statistically significant, whereas significant differences were observed in nearly all comparisons of Polish and non-Polish European populations. Phylogenetic analyses demonstrated tight clustering of Polish populations separated from non-Polish groups. Population clustering based on Y-STR haplotypes generally correlates well with the geography and history of the region. Thus, our data are consistent with the assumption of homogeneity of present-day paternal lineages within Poland and their distinctiveness from other parts of Europe, at least in respect to their Y-STR haplotypes.

O18

Bantu and European Y-lineages in Sub-Saharan Africa

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Sub-Saharan Africa ancient diversity is known to have been re-modulated to a large extent by Bantu migrations in the sub-Sahel region, in two southwards waves of advance, through both the west and in the east coasts.

Haplotype matching performed for Y-STR haplotypes in several sub-Saharan populations, inside and outside the migration path (Cape Verde, Guinea Bissau, São Tomé and Príncipe, Central African Pygmies, Angola, Mozambique and South African Bantus), allowed the confirmation of a putative founder haplotype, and its one-step neighbours, as good Bantu markers, and to detect an increasing drift towards south, with a stronger reduction of diversity along the western coast. A mixed frequency distribution for the Bantu haplotype core in South Africa relatively to the western and eastern pools, seems to be evidence for the intermingling between both Bantu waves in that region.

The proportion of male lineages considered as predating the Bantu expansion, reached 8.8% in Mozambique.

Further influence on sub-Saharan diversity could have been contributed through the colonial period; in Mozambique, the European genetic impact in the male component was estimated to be around 5.9%, in significant contrast with the female counterpart, where no European lineages were detected.

O19

Going on-line with Y-STR Testing: The BCA Laboratory Experience

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To comply with the US National Standards (DNA Advisory Board, 1998), both developmental and laboratory specific internal validation studies must be completed prior to using a new technology on forensic casework. The BCA Laboratory worked in conjunction with Reliagene Technologies to complete validation studies using the Y-Plex™ 5 and Y- Plex™ 6 kits. These two kits allow for the amplification of the following loci: DYS19, DYS385, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS438 and DYS439. There is one overlapping locus (DYS389II) between the two kits for QC purposes. Results to be presented include data from the following studies: 1) non-human and female DNA 2) environmental insult 3) sensitivity 4) adjudicated casework and 5) population data from 300 Minnesotans including Caucasians, Hispanics, African Americans and Native Americans. Results from these studies are being used to develop a casework approach for analysis and interpretation of Y-STRs at the BCA Forensic Science Laboratory.

O20

How important are local differences in autosomal and Y-Chromosome STRs frequencies?

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Introduction. Allele frequency distributions of many autosomal STR do not show great variations within large ethnic groups (i.e., caucasian). Thus, similar conclusions can usually be drawn whether using a general reference database or a local one. However, that may not be the case for Y-chromosome markers, which may reveal evidence of population differentiation even at the micro-geographical level in small rural areas. In the present study we used a Bayesian approach to address the issue of the possible relevance of those differences for the interpretation of DNA profiles.

Methods. We studied male subjects living in three areas of Cantabria, a region in Northern Spain: the densely populated and well communicated coastal area, which may be considered as the region's reference population; and two relatively isolated mountainous areas, the Pas and Liébana Valleys. DNA was isolated from peripheral blood. Nine autosomal STRs (Profiler Plus kit) and 5 Y-STRs (DYS19, DYS389-I, DYS389-II, DYS390 and DYS393) were amplified by PCR and alleles identified by using a ABI310 analyzer. Paternity indices were estimated from autosomal STR data of virtual trios composed by randomly selected individuals and their possible offspring. Matching probabilities were also calculated for each Y-STR haplotype.

Results. Allelic frequencies were significantly different for 3 out of 9 autosomal STRs studied, with an overall Fst value of 0.3% (p=0.001), the greatest difference being between the Pas Valley and the coastal area. Using allelic frequencies from the coastal general database resulted in a slight overestimation of paternity indices, but it was of no practical importance, over a wide range of *a priori* probabilities.

More marked differences were found regarding Y-STR alleles, with an overall Fst value of 3%. Using coastal database as the reference, instead of the specific valley database, generally underestimated matching probabilities, and subsequently resulted in higher likelihood ratios. In 37% of Pas Valley cases and in 13% of Liébana cases the difference was 10-fold or higher. We used a Bayesian approach to analyse the relevance of those differences in different scenarios. When *a priori* probabilities were neutral or high (i.e., 50% or higher), little difference existed among *a posteriori* probabilities, whether using the general database of haplotype frequencies or the specific one. However, with low "a priori" probability (i.e. 10%), *a posteriori* probabilities were rather different in many cases, depending on the database used as reference.

Conclusion. The European Y-STR database reveals a relative homogeneity in allelic frequencies. However, this study suggests that, in relatively isolated areas, not taking into consideration Y-STR specific frequencies may result in interpretation errors when Y-chromosome haplotypes are used in forensic cases, particularly if *a priori* odds of suspicion are low.

O21

The Design and Compilation of a US Y -STR Haplotype Reference Database

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The establishment of a US National Y-STR reference database is essential to facilitate the generation of reliable estimates of Y-STR haplotype frequencies. Such haplotype frequencies are required to provide a statistical estimate of the significance of a match. A US Y-STR Haplotype Reference Database has been created by the International Forensic Y-User Group and is maintained by the Max Plank Institute for Evolutionary Anthropology, Leipzig, Germany. Currently, however, this database is limited to a set of 9 core Y-STRs which may reduce its operational usefulness in certain circumstances, particularly in light of the development of Y-STR multiplexes consisting of over 40 different loci. Y-STR loci, unlike traditional STR markers, are not independent of one another and are co-inherited as extended haplotypes of linked markers. The estimation of the frequency of occurrence of a particular haplotype therefore necessitates the use of a counting method, which is dependent upon the size of the database.

This presentation will describe in detail the design and establishment of a comprehensive on-line Y-STR database, the aim of which is to type all potentially useful Y-STR markers in a variety of geographically and ethnically diverse populations.

The database records initially comprise data generated in our laboratory based upon a 19 Y-STR locus extended haplotype. The loci tested include DYS19, DYS385 (a) and (b), DYS388, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS425, DYS434, DYS437, DYS438, DYS439, Y-GATA-C4, Y-GATA-A7.1, Y-GATA-A7.2 and Y-GATA-H4. We have compiled data from various Caucasian, African American and Hispanic populations. Although some (unpublished) data exists for some of these loci in US populations it is not readily accessible to the crime laboratory community and usually does not contain 'extended' haplotype data due to the technological restraints of the systems employed by the investigators. A key component of the strategy is to allow for the continuous updating of haplotype data using the same samples. This ensures that as new markers are developed the same samples would be re-typed and a new extended haplotype developed. Thus, any laboratory needing haplotype data for any combination of Y-STR markers would be served. We have enlisted the aid of geographically diverse crime laboratories to obtain the necessary samples. In exchange for the samples the crime laboratories benefit by obtaining a custom built no-cost local Y-STR database.

Our plans to extend the database to include an additional 42 loci will also be described.

O22

Mutations at Y-STR loci: a study of 1767 father-son pairs of Norwegian origin

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We present findings of individual mutation rates in 9 Y-STR loci (DYS19, DYS385, DYS388, DYS389 I, DYS389 II, DYS390, DYS391, DYS392 and DYS393) in a total of 15 903 male germline transmissions from father/son pairs of confirmed paternity at five minisatellite loci which are commonly applied for paternity testing. A total of 37 mutations were identified. There was an inter-locus heterogeneity in mutation rates ranging from 5.7×10^{-4} to 5.1×10^{-3}. The overall average mutation rate was 2.3×10^{-3}. In one father/son pair, mutations at two Y-STR loci were observed. Of 37 mutations 23 were gains (22 single-step mutations and 1 double-step mutation) and 12 were single-step losses. Two mutations in the complex DYS389I/II locus (which consists of four repeat motifs) involved changes in two and three motifs respectively. Sequencing results will be presented and several potential factors which may contribute to the observed differences in the evolutionary dynamics of theses microsatellites will be discussed.

O23

Y chromosome diversity in Kurds and Assyrians living in Armenia

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734 ethnic Armenian, 196 Kurd and 106 Assyrian men were sampled in Armenia. DNA was extracted from buccal swab and typed for six STR and 12 Unique Event Polymorphism (UEP) loci mapping to the nonrecombined portion of the human Y chromosome. The Armenian sample was divided into 6 regions according to the paternal grandfather's birthplace (Weale et al., 2001). Kurds and Assyrians were each considered as single ethno-territorial groups. UEP markers defined 8 haplogroups (hg) for the Armenian population and 7 haplogroups for Kurds and Assyrians. All 8 regions differed significantly ($p<0.001$) from each other according to the frequency of haplogroups. UEP gene diversity (h) varied from 0.599 in Armenians from the northern region to 0.673 in Kurds. Genetic diversity (based on UEP+microsatellite haplotype frequencies) in Kurds (0.942+/-0.006) is significantly lower ($p<0.01$) than in all Armenian regional subpopulations except the southern (Syunik) region. The same index in Assyrians (0.954+/-0.008) is also significantly lower ($p<0.05$) than in all Armenian groups except the Syunik region. Kurds and Assyrians did not differ significantly in their UEP+microsatellite haplotype gene diversity values. Analysis of Molecular Variance (AMOVA) revealed that 96.86% of the variation at the six microsatellite loci is due to the differences between individuals, whereas differences between three ethnic groups and between 6 regional Armenian populations accounted for 2.09% and 1.05% of the variation respectively. Pairwise exact test for population differentiation (Raymond and Rousset, 1995) showed significant differences ($p<0.001$) between Assyrians and all Armenian regional populations, and also between Kurds and all Armenian regional populations. Assyrians and Kurds also differ significantly ($p<0.001$) from one another according to pairwise exact tests. Overall, Assyrians and Kurds appear to be genetically distinct from the general Armenian population, with F_{st} values suggesting that Assyrians are the most differentiated group from all Armenian regional populations and from Kurds.

O24

Evolutionary history based on seven loci Y-Chromosome STR haplotypes of 14 Amerindian tribes of the Amazonian and Orinoquian regions of Colombia.

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We have determine the genetic structure and evolutionary history in 157 individuals from 14 Amerindian tribes of Colombia belonging to four linguistic families Arawak (Curripaco and Piapoco tribes), Macú-Puinave (Puinave and Nukak tribes), Guahibo (Guahibo and Guayabero tribes) and Tucano (Cubeo, Desano, Piratapuyo, Tatuyo, Tucano and Wanano tribes) based on 7 loci Y-chromosome STR haplotypes (DYS19, DYS389-I, DYS389-II, DYS390, DYS391, DYS392 y DYS393). A total of 59 haplotypes were identified with a haplotype diversity of 0.9553. The most frequent haplotype was H29: 13,12,30,24,10,15,13 (14%); followed by H17:13,12,30,23,10,15,13 (8.92%); H45:13,13,30,24,10,14,13 (8.3%); and H10:13,14,32,24,10,15,13 (5.73%). A comparison of the Amerindian haplotype with the Caucasian Mestizo and Afro-Colombian populations showed that only 2.75% of the Amerindian haplotypes were shared with these ethnic groups.

A median network analysis for the entire continent was carried out in order to determine the Ancestral haplotype as well as the most recent common ancestor (time of entry into America) for the Amerindian population. This analysis included a total of 465 individuals from 35 Amerindian, Na-Dene and Skimo-Aleutian populations described in the literature. The ancestral haplotype found was H45:13,13,30,24,10,14,13 and the time of entry into the continent was 22300 ybp (15695-28905 ybp) corroborating previous findings based on archeological data and mtDNA analysis.

The AMOVA showed that 36% of the genetic differences were due to differences between groups ($\Phi st = 0.3672$, $p<0.00000$), a result likely due to genetic drift. In addition 25% of the genetic variation was due to differences in linguistic affiliation. We have correlated the genetic data with the geographic and linguistic classification using similarity dendrograms, Mantel test, and Multidimensional Scaling analysis. The results indicate that the Amerindian tribes have evolved in the genetic, linguistic and geographic aspects in a highly correlated fashion.

Thus, Y-STR haplotypes represent a powerful tool for anthropological studies in order to reconstruct the evolutionary history of human populations.