O1

Applications of 5-Dye Technology in Forensic DNA Typing and Analysis

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In the past decade, fluorescence based DNA detection systems have been widely used in forensic DNA analysis. Fluorescence detection methods have greatly aided the sensitivity and ease of measurement of PCR amplified short tandem repeat (STR) alleles. In the new multiplexed STR genotyping kits, fluorescent dyes are covalently coupled to primers for each locus. Fluorescence measurements involve detecting light emitted from an excited dye at a specific wavelength. Filters are used to select fluorescent dye signals at optimal wavelength ranges. In ABI PRISM® instruments, fluorescent signals are separated by a diffraction grating and projected onto a charge-coupled device (CCD) camera during data collection. Multicomponent analysis is performed using a matrix that excludes the contribution of neighboring dyes.

Earlier kits from Applied Biosytems (AmpF*l*STR®kits) used PCR primers with NHS-ester dyes 5-FAM™, JOETM or NED™ and ROX™ dyes and emission spectra ranging from 522 nm to 607 nm. More recently, Applied Biosystems has developed a unique five-dye technology for automated DNA fragment analysis. The introduction of the new 5-dye chemistry involves replacement of 5-FAM with 6-FAM™, JOE with VIC™ and ROX with LIZ™ and incorporation of the new PET™ dye into the existing system. The five dyes 6-FAM, VIC, NED, PET and LIZ expand the spectral detection range on the ABI PRISM instrumentation to 660 nm. The addition of more dyes thus enables more loci to be multiplexed into a single PCR amplification. Additionally, the new dyes will increase genotyping throughput significantly, since more loci can be analyzed simultaneously in a single lane or capillary. One advantage of this arrangement is that minimal hardware changes to existing instrument platforms is necessary. Examples of use of this technology in Applied Biosystems kits will be presented including the Identifiler™ kit (15 loci and Amelogenin) and other kits in development (e.g. SNP detection). The performance of the 5-dye technology across the ABI PRISM 310, 377, 3700 and 3100 genetic analyzers will be presented. Also, reliable signal intensity, and software analysis compatibility with the new expanded 5-dye set will be demonstrated.

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O2

Rapid preparation of SNP multiplexes utilising Universal Reporter Primers, and their detection by gel electrophoresis and microfabricated arrays

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We describe a method that enables the rapid production and evaluation of multiplexes. Two kinds of primer are employed. Initial SNP detection and production of unique, allele-dependant products is initiated by locus-specific primers. Two forward primers and one reverse primer are required for each biallelic SNP locus under investigation. Each locus-specific primer consists of a 3' region which is 16–27 bases in length and is complementary to the target DNA sequence. The 5' region of these primers consists of 20-base, non-complementary tag sequences termed the universal sequences. Three different 20-base universal sequences are employed. One sequence is used for one of the forward primer pair, a second sequence for the other forward primer and a third sequence for the reverse primer. All of the reverse primers bear the same universal sequence at their 5' regions.

In addition to the different universal sequence present at their 5' end , the forward primers also differ at their terminal 3' base. This terminal 3' base is complementary to the base present at the SNP site on the target DNA. During the annealing phase of the PCR cycle either of the two forward primers may anneal to target DNA at the site of the SNP. Extension and product formation will only occur however, if the forward primer has the correct, complementary terminal 3' base (ARMS principle). Amplification with these primers results in the formation of 2 products which are of equal size but which differ in the universal sequence of the 20 bases present at their 5' end. Only one of these products is produced with DNA from individuals who are homozygotic at the SNP site. Both products are produced from heterozygotes.

Also present in the PCR reaction mix is a high concentration of two 20-base universal primers. These universal primers are identical to the sequence of the 20 bases present at the 5' end of the forward locus-specific primers. One of these universal primers is labelled with FAM dye and the other primer is labelled with JOE dye. These dyes can be distinguished with a P/E 377 Gel Sequencer. The universal primers cannot produce PCR products from template DNA directly, however they can amplify any product produced by a locus-specific primer which bears the correct universal sequence of bases at its 5' end.

We have developed multiplexes that can be used on a variety of different platforms – these include standard gel electrophoresis and microfabricated arrays. We have evaluated more than 80 SNPs available from public SNP databases and tested these with samples from 3 different populations to estimate levels of polymorphism within each ethnic group. New multiplexes (10–13 SNPs) have been routinely produced and we are currently optimising the production of much larger multiplexes.

We have demonstrated that SNPs are much better to analyse very degraded samples (compared to STRs) because the fragment length is much smaller (<100 bases).

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03

A novel DNA microarray system for analysis of limited forensic evidence material

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The ability to match a genetic profile from an evidence material with genetic profiles of suspects makes an important contribution to the traditional criminal investigations. Furthermore, the possibility of analyzing very small amounts of DNA found at the scene of a crime has greatly influenced forensic analysis. Today mitochondrial DNA analysis is widely used on biological samples of limited amounts because of the high copy number of mtDNA. However, its discriminating power is not as high as in analysis of nuclear DNA, wherefore a method that combines mitochondrial and nuclear markers would be a powerful tool to reduce the probability of DNA identity by chance for limited DNA samples. Analysis of DNA from material of bad quality and small amounts are also often time-consuming and difficult to perform. In order to increase the discrimination power and reduce the turnaround time in analysis of samples with minute DNA amounts, we have developed a one color microarray-based SNP detection system.

The system relies on minisequencing in solution prior to hybridization to universal tag-arrays. The minisequencing reaction is based on the extension of a tagged primer with a chain terminating fluorescent dideoxynucleotide at the position to be interrogated. Our one color system detects C and T polymorphisms in separate multiplex reactions with the fluorophore TAMRA coupled to the respective dideoxynucleotide. The extended primers are hybridized through its complementary tag sequence on the array and positive signals are detected by a confocal laser scanner (Scan Array 5000, Packard BioScience). The tag-arrays are produced with an in house robotic workstation (GMS 417 Arrayer, SDS) using silylated slides with aldehyde chemistry. In order to maintain a high sensitivity even for analysis of the nuclear markers has the PCR systems been designed to amplify very short fragments.

A first chip to be evaluated contains 13 nuclear and 21 mitochondrial SNP markers. The mitochondrial markers cover polymorphisms within the coding region as well as in the hypervariable HVI and HVII regions of the mitochondrial genome. The markers have been tested and evaluated together on microarrays on a number of control samples with known genotypes as well as on a few forensic samples. This microarray based forensic analysis has proven very reliable, sensitive and rapid. A fully developed system will make it possible to perform large, rapid and cost effective analyses with high discrimination power.

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04

Automated SNP Genotyping by MALDI-TOF Mass Spectrometry

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In the post-genomic era, one of the next important steps towards understanding and curing human diseases is the establishment of genotype-phenotype correlation due to gene specific polymorphisms. Single nucleotide polymorphisms (SNPs), the most common type of genetic variations, have been shown to play a major role in the context of genetic diseases, pharmacogenetics, drug development and forensics. For monitoring these kinds of genetic diversities technologies are required capable of genotyping many thousands of individual samples per day, as well as dynamic software and database systems for translating the results of the measurements to useful knowledge that can be implemented in diverse settings.

Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) based systems can be utilised for genotyping and disease management applications. Successful implementation of MALDI-TOF MS for fully automated SNP genotyping has required development of advanced molecular biological assay systems, sensitivity increasing sample preparations as well as dynamic software modules for measurement controlling and data interpretation.

As practical examples we present singleplex and multiplex genotyping of several polymorphisms with diagnostic relevance. Analytical strategies for generation and MALDI-TOF MS measurement of allele specific products are shown as well as computer based automated allele calling and genotype assignment.

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O5

Genotyping for SNPs using a Multiplex detection assay

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Single nucleotide polymorphisms (SNPs) are very abundant and highly conserved in the human genome (i.e. average of 1 every 1000 nucleotides). These mutations have been shown to have application in a variety of genetic studies including human identification. Numerous methods and instrumentation to perform SNPs genotyping have been described, however, very few of them can be used to perform high volume genotyping of large number of SNPs in an efficient and cost effective manner.

A simple multi functional analytical system developed by Luminex Corp. makes it possible to simultaneously analyze multiple analytes. The components of this system are a flow sorter fluoroanalyzer instrument and up to 100 uniquely labeled fluorescent microspheres. The instrument contains 2 lasers, one to identify each microsphere class and a second to quantify the amount of fluorescently tagged reporter molecules bound to it. Microspheres can be chemically coupled with DNA. For example, for the detection of different DNA sequences, each microsphere class may be coated with a different sequence specific oligonucleotide probe and hybridized to a mixture of labeled DNA sequences.

In the assay developed in our laboratory, biotin-labeled PCR primers were used to generate single-stranded DNA fragments spanning one or more SNPs in a DNA sample in multiplex PCR reaction. For each SNP, a probe (~19 bp) complementary to the SNP sequence was synthesized and covalently attached to each class or type of fluorescent microspheres. The different types of microspheres, each containing a different probe, were combined. The mixture of microspheres-probes was hybridized to the biotin-labeled single-stranded DNA. After 30 minutes hybridization, Streptavidin-conjugated phycoerythrin was added to label the DNA and the reaction placed in the Luminex flowcytometer for analysis. The instrument measured the median fluorescent intensity of each bead type and this information was used to determine the presence or absence of each SNP sequence in the amplified sample. Accuracy and sensitivity of the assay was confirmed by comparing genotypes obtained with these assays with those generated by SSP. Typically, this assay discriminated the two alleles in a biallelic SNP with a signal to noise ratio >10 to 1.

The SNPs used for these assays were selected based on several criteria such as, chromosomal localization, robustness of the amplification assay and allele frequency in different populations.

The total time required for this assay, after DNA isolation, was less than three and half hours and the results may include the reaction information of up to 100 probes per sample. These results demonstrate that the fluorescent microsphere-based SNP genotyping assay is a sensitive and reliable high-throughput assay that may significantly reduce the reagent and labor cost of this type of assay.

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O6

Typing Y chromosome SNPs with DNA microarray technology

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We are developing DNA microarrays for Y chromosome SNP typing in forensics using the Affymetrix 417 microarrayer and the microarray scanner Affymetrix 418. The advantages of using Y chromosome SNPs include the low mutation rates (in deficiency paternity testing), the sensitivity of Y chromosome SNP typing in mix stains when the male component is in a very low proportion and the possibility of using high-throughput methods for typing.

A total of 32 Y chromosome SNPs, distributed in 4 PCR multiplex were selected including the most interesting ones in Western European populations. The SNPs selected include some classical ones and some recently reported Y SNPs (Underhill et al., 2000).

Low density DNA microarrays are sufficient for forensic applications. Spotting probes in slides, followed by Southern hybridization with PCR products labeled with Cy3 and Cy5, are in general adequate for SNP typing. Different spotting systems are available and the pin-and-ring system that we have selected is robust and reproducible.

Evaporation has its greatest potential impact in the samples in the wells waiting to be spotted and its control is the main problem for reproducibility.

The type of slides used, hybridization strategies and typing strategies will be shown and discussed. Orientated superaldehyde substrates have clear advantages over non-orientated ones.

The advantages and disadvantages of the methods used to increase typing accuracy are discussed as well as the PCR multiplex strategies which must necessarily be combined with microarray technology, at least in criminal casework applications.

Underhill PA et al. Y chromosome sequence variation and the history of human populations. Nat Genet. 26(3):358-61 (2000)

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07

The differential slave trade to Europe and Brazil from the western and eastern African coasts as registered in the mtDNA pool

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The Atlantic slave trade began in the fifteenth century with the European overseas expansion. In the beginning, the main origin of slaves was the western African coast. Of a total of ~ 13,000,000 slaves, it is estimated that 23% were from Angola, 15% Slave Coast, Benin and Senegambia each, and 12% from Gold Coast. In the eighteenth century, due to the loss of Portuguese control over some of these regions, Mozambique and Madagascar became the major source of the slaves shipped, amounting to a proportion of around ~ 8% of the total trade. This shift in the place of origin of the slaves with time was accompanied by a change in the place to where they were exported: in that period, the majority of the European countries forbade the entry of black slaves, but the trade to the former European colonies in America, e.g., Brazil, was still expanding.

Until now, it has been impossible to investigate this issue using mtDNA, because there were few populations on the eastern coast of Africa characterised for mtDNA variation. We have screened 109 individuals from Mozambique, the main eastern source of slaves, and have investigated whether the contribution of Mozambican sequences to the mtDNA sequence pool of Brazil was indeed higher than to the European pool.

There was a considerable number of sequence matches between Mozambique and Brazil, representing 10 shared sequences in a total of 53 different haplotypes from African haplogroups in the Brazilian pool. Two of these matches correspond to sequences that have so far only been observed in Mozambique, 5 have not been detected in western Africa, while 3 have been observed in western Africa. All the Mozambique-Brazil matches for haplogroup L3e1 were not shared elsewhere, except with the southern Khoisan-speaking populations. With respect to the 40 Brazilian sequences with no match with Mozambique, there were 8 matches with the African database, only 2 of them restricted to western African populations.

For sequences observed in the European pool from African haplogroups, in a total of 48 different haplotypes, 4 matches were detected with Mozambique, but 3 of those sequences were also detected in western African populations. Besides those, 9 further matches were detected, of which 2 were western African specific, 1 northern African, 2 eastern and 4 were widespread.

So, mtDNA reflects a higher proportion of matches between sequences from Mozambique and Brazil compared to that between Mozambique and Europe, in accordance with the historical documentation. This is particularly striking since other documented factors would have tended to weaken this signal. Firstly, the female/male proportion of the slaves taken to Europe was much higher than to America, and secondly, slave reproduction (particularly from female slave and white owner) was stimulated in Europe (especially after the ban on the importation of slaves after the middle of the eighteenth century) but was been repressed in America.

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08

Estimating the ethnic origin of individuals using Short Tandem Repeat loci of forensic relevance

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The potential of STR loci of forensic relevance to affiliate individuals from 8 populations to their respective ethnic origin was investigated. In the first setup the discrimination between 2 Hungarian populations was tested (Budapest Caucasians and Romanies from Baranya county). In the second setup the discrimination between Europids from Austria and Arabs from Egypt was tested. Finally the feasibility to affiliate US-Americans to one of 4 possible ethnic origins (African Americans, US-Asians, US-Caucasians, and US-Hispanics) was investigated. To that end reference databases for 13 to 19 loci were established for each population. Using a likelihood ratio approach the ratios of the allelic frequencies found in the different populations were used to estimate the ethnic origin under the assumption that an individual is equally likely to originate from the respective population. For the Hungarian populations (16 STRs) it was possible to identify 91% of the Caucasians and 84% of the Romanies. Eightyfive percent of the Austrians and the Egyptians could be identified in the European/Arab setup (19 STRs). Using the 13 CODIS core loci it was possible to recognize 83% of the African Americans and 85% of the US-Asians. The discrimination between US-Caucasians and US-Hispanics was not satisfying. Nevertheless it was in 95% correct to state that an individual is either of US-Hispanic or US-Caucasian origin. Estimating the ethic origin of unknown perpetrators using STRs appears to be a promising approach to some forensic problems (for instance mass screening for a rapist) provided appropriate reference databases are used and the results are carefully interpreted.

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O9

An evaluation of the proportion of identical Y-STR haplotypes due to recurrent mutation

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Search for haplotype matching is the main tool used in forensics to evaluate how significant is the observation of a particular haplotype in a certain sample. A neglected phenomenon that can be an important bias source to this kind of analysis is the possibility of recurrence, due to the high mutation rate of STRs, and hence, the rise of haplotypes identical by state, rather than by descent.

One approach that has been used to evaluate the proportion of these classes among identical haplotypes was to assess haplotype identity by the information from SNPs, the same Y-STR haplotype in two different Y-SNP haplogroups pointing to identity by state and not by descent. De Knijff (2000) estimated a proportion of 0.2% of identity by state in a sample of 275 Dutch screened for 8 Y-STRs and 4-SNPs.

Unfortunately, combined information for both Y-STRs and Y-SNPs in the same sample is very rare, and it is therefore impossible to measure the proportion of identity by state, at a large scale, by the approach above described.

We suggest an approach based upon mismatch distributions/haplotype pairwise comparison that can at least indicate the proportion of Y-STR haplotypes in which mutation can originate identity by state. It can be subdivided in two levels of complexity. First, we determine the proportion of pairs of haplotypes that differ at one locus, since (almost) only these can become identical by state. Second, we calculate the proportion of pairs of haplotypes that differ only by a single repeat unit, since it is known that most mutations in STRs are single step.

We applied this approach to the sample of 229 Dutch (87 Holland, 44 Friesland, 48 Groningen and 50 Limburg; 123 different haplotypes based on the following loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393) published in the Y-STR Haplotype Reference Database (http://ystr.charite.de). Values of 7.35% and 6.38% were obtained for pairs of Y-STR haplotypes that differ at one locus and at a single repeat, respectively. These two values show that 86.8% of the differences between haplotypes differing in one locus are of a single repeat unit. Per generation, the probability of a certain Y-STR haplotype becoming identical by state to another one inside the class of those that differ by a single repeat is thus seven times the product of the proportion of this class by the mutation rate for a Y-STR locus (mean of 3.17×10^{-3}), which results, for this sample, in a value of 0.14%. This value is in close agreement to the one observed by De Knijff (2000), although not considering the rare cases of identity by state produced by more complex, namely multigenerational, phenomena.

It seems therefore that this approach can be useful in the forensic field, for the evaluation of the significance and evidential value of Y-STR haplotype matches.

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O10

Micro-geographic patterns of highly informative Y chromosome haplotypes (using biallelic markers and STRs) in Galicia (NW Spain): forensic and anthropological implications

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The effect of sampling in the estimation of Y chromosome haplotype frequencies is analyzed in this work in the population of Galicia (NW Spain) as well as the importance of population substructuring at local level.

Databases are collected on the basis of unrelatedness and consequently frequencies could be underestimated because of deliberate non-relatedness selection criteria. In a random survey from a large cosmopolitan population with marked mobility, the chance of selecting two patri-lineage related people is low. However, in small isolated rural populations the effect may be important and must be considered if the frequency estimate from a general population is used to make inferences on the former.

Galicia (NW Spain) comprises a population of nearly 3.000.000 inhabitants. Two thirds of the population live in villages of 1.000 to 10.000 inhabitants.

Two population surveys were carried out, one (80 individuals) from general random population (mainly from the most important cities of the area) and another, excluding the main cities with a total of 292 individuals distributed in 7 natural geographic areas.

7 Y STRs and 10 biallelic markers were analyzed allowing the construction of highly informative haplotypes. Significant differences were found for Y chromosome SNPs between the population living in towns and that in rural areas.

Surprisingly there are no significant differences among the different geographic natural areas with low Fst values.

The isolation of this region from the rest of Spain and Portugal and the high emigration rates together with almost no inmigration has preserved the cultural identity of this region. The possible effect of cultural traits on this unexpected microgeographic distribution of Y chromosome haplotypes is also discussed in this work.

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O11

Comparison of Y Chromosome Haplotypes in Three Racial Groups and the Possibility of Predicting Ethnic Origin

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Currently most STRs found on the Y-chromosome exhibit much lower levels of polymorphism when compared to autosomal STRs. However, unlike autosomal STRs, they often show marked differences in allele frequency distributions between racial groups. 250 unrelated individuals of UK Caucasian, UK Afro-Caribbean and UK Asian (from the Indian sub-continent) origin were typed with 11 Y-STR loci. The resulting haplotypes were compared between racial groups and the differences observed are presented. A supplementary protein system (Gc), known to be a useful race related marker in the Afro-Caribbean population, can be used to improve racial discrimination between this and other racial groupings.

A blind trial was conducted to assess the validity of predicting ethnic origin from an unknown DNA sample and a case is included to illustrate where this technique has been usefully applied. We conclude that this set of tests could provide a useful starting point in the quest to produce a simple method of providing a probability of ethnic origin from an unknown DNA sample.

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O12

Population Studies on 17 STR Loci Routinely Used in Forensic Analyses

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The short tandem repeat (STR) loci typically used in the United States for forensic analyses are CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. These 13 STR loci are the core loci for the U.S. national DNA databank CODIS (Combined DNA Index System). When a match occurs between the DNA profiles of the evidentiary sample and the reference sample from a suspect (or a victim, depending on the circumstances of the case), an estimate of the rarity of the evidentiary DNA profile is made. The computation is based on using the product rule with adjustments for population substructure. The National Research Council (NRC) II Report recommends Wright's FST statistic (or θ) to estimate the degree of relatedness in a population and to correct for departures from Hardy-Weinberg expectations due to population substructure. The NRC II Report recommended that a conservative value for θ is 0.01, although the NRC II Report suggested that 0.03 may be used until more data were collected or for more genetically isolated populations, such as Native Americans. Thus, allele frequency data and θ estimates have been generated.

Data on the CODIS STR loci are available on more than 50 sample population groups comprising the five major population categories. The value of θ for most population groups is much lower than 0.01. For African Americans θ is 0.0006; for U.S. Caucasians θ is -0.0005; for Europeans θ is 0.0028; for Hispanics θ is 0.0021; for far east Asians θ is 0.0039; and for Native Americans θ is 0.0282. A θ value of 0.0090 was estimated for nine of the thirteen core STR loci in Chamorros and Filipinos. The data support that the degree of relatedness in the African American, Caucasian, Hispanic and Asian groups is low. In fact, the Hispanic value is likely inflated because southeastern and southwestern Hispanic data sets were pooled for the FST calculation. Typically, these two groups are not merged for profile frequency estimates (although these data suggest that pooling is possible). The F_{ST} value of 0.0282 for Native Americans is consistent with expectations for more isolated groups. The FST values estimated from these data support the generalized recommendations of the NRC II Report for taking a value of 0.01 as a conservative threshold for most population groups and the value of 0.03 for Native Americans as reasonable.

Because of the defined requirements for core STR loci, manufacturers have developed megaplex kits that enable simultaneous amplification of the 13 CODIS STR loci in a single PCR. The AmpFlSTR¨ Identifiler™ PCR Amplification kit (Applied Biosystems, Foster City, CA) provides reagents for simultaneous amplification of the 13 core CODIS STR loci, two additional STR loci (D2S1338 and D19S433) and the amelogenin locus. The PowerPlex¨ 16 kit (Promega Corp., Madison, WI) provides reagents for simultaneous amplification of the 13 core CODIS STR loci, two additional STR loci (Penta D and Penta E) and the amelogenin locus. Population data on the additional four STR loci (D2S1338, D19S433, Penta D, and Penta E) show that 1) these loci are highly polymorphic in all population groups studied; and 2) the θ estimates are well below 0.01. Thus, the additional STR loci in the megaplex kits will also be useful genetic markers for forensic applications.

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O13

Genotyping of 9 STR systems in combination with 11 diallelic polymorphisms on the Y-chromosome by fragment analysis and minisequencing

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The study of Y chromosomal haplotypes and their relationship to human evolution and variation is increasing rapidly in the fields of anthropology and forensic genetics. Although autosomal STRs are commonly used and very informative for paternity testing and forensic identification, the use of informations from the nonrecombining portion of the Y chromosome is important and provides additional data in cases when the offspring is a male or for mixed male/female crime stains. For this purpose in the past two years more and more attention has been paid to the examination of diallelic polymorphisms (SNPs) on the Y chromosome.

Many techniques exist for the detection of sequence variations. For detection of SNPs a commonly used method is the digestion of amplified PCR products with restriction enzymes followed by agarose gel or polyacrylamide gel electrophoresis, which is very time consuming and expensive.

Here, we describe an approach for typing of 10 Y chromosomal diallelic polymorphisms (SNPs) using the single base extention technology (minisequencing). The method requires 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) as templates. The minisequencing reaction is then performed in a single tube with the commercially available SNaPshot-Kit from Applied Biosystems and needs only one run per sample in an ABI-310 genetic analyser.

The amplified DNA fragments generated by the PCR are ranging from 106 to 363 base pairs in length. Therefore it should be possible to use this method also when the DNA sample contains partially degraded DNA extracted from forensic case work samples.

The present study was performed to examine the frequencies and haplotype relationships of the above mentioned diallelic polymorphims in a sample of 100 individuals of Caucasian origin. In addition to the 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.

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O14

Y chromosome Short Tandem Repeat polymorphisms: A comparison between humans and chimpanzees

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With the development of the PCR techniques, during the last twenty years the STRs became the most studied markers in population and forensic genetics.

The high number of STRs widespread all over the genome, the high levels of polymorphism and the simplicity of the typing methods, were responsible for a large accumulation of STR population databases. The importance of the establishment of a nomenclature was already emphasised by different groups working both in forensic and population genetic fields (ref.). It is crucial for data comparison that all the laboratories working with the same STRs use the same nomenclature. In the application of STRs to population and forensic studies it's also important the definition of the mutation rates. In most of the works concerning this matter, there is a general opinion that the STR mutation rates are dependent on the locus structure and the number of repeat units of the alleles (Ellegren, 2000; Xu et al., 2000). However, those conclusions are only valid if the nomenclature actually reflects the locus composition.

Concerning the Y-specific STRs, there was a change in the nomenclature originally introduced for DYS19, DYS389 and DYS390, since it was found that some repetitive motifs, originally considered as invariant, were in fact polymorphic in some populations. These nomenclature changes, although necessary, are sometimes prone to some confusion on the comparative analysis of data sets produced by different laboratories.

Ayub et al. (1999) and White et al. (2000) recently described many new Y-chromosome markers and more are expected in the near future.
The aim of this work was to make a comparative analysis of the sequence structure of the Y STR loci (DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, GATA A10, GATA A7.1, GATA A7.2, GATA C4, and GATA H4) in Humans and in Chimpanzees, in order to better elucidate the structure and stability of these STRs.

Primers described for Y GATA A4 were found to amplify the same region as reported for DYS439. Moreover, the GATA A4 forward primer only matches the repeat flanking region in 14 of the 28 bp, being responsible for a very weak amplification. Therefore it was not included in this study.

The analysis of the repeat and sequence structure observed in chimpanzee and human Y-chromosomes allowed evolutionary comparisons as well as a sounder basis for establishing STR nomenclature criteria.

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Allelic diversity and mutation at the hypervariable minisatellite locus DYF155S1 (MSY-1).

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In a Norwegian population material the allele length variation at locus DYF155S1 was studied in 160 unrelated males. Thirty three different alleles were identified, and generally the alleles showed a bimodal length distribution with one group in the range 64-85 repeats and one group in the range 87–95 repeats. The highest allele freqency was found in 76 repeat alleles (14%) and the 74 and 75 repeat alleles (12% each), while all other alleles had allele frequencies less than 10%.

In 85 alleles further characterized by MVR-PCR analysis, there were a total of 78 different types. Except for two alleles with 0-repeat variants within the repeat array, the alleles consisted of 1, 3 or 4 repeat variants only. The distribution of blocks with different repeat variants along the repeat array (modular structure) might be used to group alleles. Sorting the alleles by both allele length and modular structure showed that there was a clear association between a given modular structure and the size of the allele, leading to three distinct allele length/modular structure subgroups.

Another selected material consisted of two groups of males (21 and 6 individuals) characterized by sharing one out of two Y-haplotypes (by bi-allelic markers and Y-STR's). These alleles showed a reduced amount of length and modular structure variation in locus DYF155S1 within the groups, supporting that alleles in each of these two groups have a close recent ancestry.

Allele length mutation in DYF155S1 was studied by identifying de novo mutations in a paternity case material of 1071 father/son pairs. PCR-amplified alleles from these pairs were analysed by agarose gel electrophoresis to reveal allele length mutations. A total of 27 mutations were detected with this method, giving a mutation rate about 2.5% (95% CI 1.6%–3.5%). Seventy eight percent of the events were one repeat changes with an average change of 1.5 repeats in all the 27 events. In most cases (89%) the modular structure was not changed while new repeat variants (0-repeats) were revealed in one of the mutant alleles.

The high variation revealed at this locus is in agreement with previous studies. The subgrouping of the 160/85 alleles in the population material probably reflects that the present population comprise different subpopulations sharing a closer common ancestry. Even if less than in the population material, the considerable variation within the two samples of males identical in bi-allelic and Y-STR's shows that DYF155S1 might be a powerful tool to subgroup or differentiate males in very closely related populations. The measurements of the mutation rate together with the observed mutation profile might provide a basis for statistical methods using variation in DYF155S1 to estimate age of, or relationship between, similar Y-haplogroups.

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Male/female DNA mixtures: a challenge for Y-STR analysis

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At present PCR multiplexes consisting of a set of autosomal short tandem repeats (STRs) combined with the gender determination system amelogenin are used routinely worldwide for forensic DNA analysis. For special applications, e.g. for investigation of sexual assault cases, STR loci from the Y chromosome (Y-STRs) may become the markers of choice, because these loci provide a male-specific DNA profile.

Semen traces are common in forensic casework, but their identification is not always successful, especially when a small amount of semen is mixed with a big amount of the victim's cells. As most victims are females, the analysis of male specific Y chromosome STRs is of great forensic importance. The analysis of STR loci from the Y chromosome improved the chances of being able to detect small amounts of male DNA against a high level background of female DNA decisively. Additionally the male specificity of Y-STRs may facilitate mixed stain interpretations. To take advantage of these features of Y-STRs, PCR multiplex reactions for forensic casework have to be optimised, because an unfavourable of male/female DNA leads to locus-unspecific amplification products.

The occurrence and intensity of these artefacts correlates with the amount of autosomal DNA in the mixture, the template quality, the PCR reaction conditions and the primer sequences. Especially the primers of the highly polymorphic locus DYS385, which improves the discrimination power of the Y-STR analysis significantly, are known to produce high background noise, making an interpretation of the DNA profile difficult or even impossible.

In this study we present two PCR multiplexes, which were optimised for their usefulness in casework applications, combining the Y-STR loci DYS19, DYS385, DYS392 and DYS393 (multiplex1) as well as DYS389I, DYS389II, DYS385, DYS390 and DYS391 (multiplex2). The overlapping locus DYS385 acts as internal standard for quality control purposes. Fluorescently labelled primers were taken for detecting and analysing of the amplification products on the ABI310 Genetic Analyzer.

The aim of this study was to develop PCR multiplexes for the identification of a complete 8 locus Y-haplotype with an amount of at least 200pg male DNA even against a high level female DNA background. To test the specificity and sensitivity of the PCR assay artificial mixtures of male and female control DNA were used, the mixing ratios ranging from 200 pg and 1 ng male DNA to 20 ng and 100 ng female DNA respectively.

The optimisation procedure included the following points: (1) new design of the primers for the loci DYS389, DYS390 and DYS391 in order to improve the specific quality of the PCR reaction and to reduce the length of the amplification products, (2) optimisation of the thermocycler protocol, (3) application of different commercial DNA polymerases and (4), addition of different PCR enhancer substances.

The use of PCR enhancers resulted in some effects worth mentioning. The Y-STR profiles of the DNA mixtures obtained after amplification using an assay without PCR enhancer shows a high background noise. A number of these artefact peaks is well outside the range of the STR loci. However, some artefacts mask positions in DYS385 and DYS392, preventing reliable allele identification. Varying the conditions of the PCR buffer by adding different enhancer substances results in a complex picture of increased or decreased peak heights of both artefacts and Y-STRs, which shows the strong dependence of amplification efficiency on the PCR conditions when working with DNA mixtures. Good results were obtained using the PCRx Enhancer System (Life Technologies; GibcoBRL). An increasing amount of this PCR enhancer added to the PCR assay resulted in a significant reduction of artefact peaks. All artefact peaks within the allelic range of the Y-STR loci disappeared, whereas the peak height for the Y-STRs remained constant or decreased but slightly. Artefact peaks outside the range of alleles were significantly reduced to a tenth of the original height.

Two casework examples are presented, for which the optimised Y-STR multiplexes were used with success. In both cases the male component was not interpretable with autosomal STRs; in one case not even the Y-amelogenin peak was visible.

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O17

Highly Multiplexed Assays for Measuring Polymorphisms on the Y-Chromosome

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The Y chromosome has long been recognized as a useful tool in forensic investigations, human identity testing applications, and human migration and evolutionary studies. In recent years, a number of polymorphic short tandem repeat loci have been discovered. However, due to the fact that no recombination occurs along the vast majority of the Y chromosome, the product rule cannot be used to increase the power of discrimination as when using multiple autosomal markers on separate chromosomes. Thus, the most efficient way to obtain higher levels of discrimination between unrelated samples is to invoke multiplex PCR assays where independent sites that may be widely separated on the Y chromosome are simultaneously targeted. As the literature contains references to Y STR multiplexes that at most only amplify five or six loci simultaneously, we have made an effort to increase the level of multiplexing for Y STRs. We have successfully demonstrated several 10-plexes and will present work from a megaplex capable of simultaneously amplifying more than 17 different Y STR markers. Our megaplex includes all of the Y STRs that make up the minimal haplotype used in Europe (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, and DYS393) plus additional polymorphic Y STRs, such as DYS437, DYS438, DYS439, GATA A7.1, and GATA H4. We are also developing single nucleotide polymorphism assays from the Y chromosome that may be rapidly analyzed with time-of-flight mass spectrometry. With improved multiplexes and techniques for more rapid data collection such as mass spectrometry, Y chromosome haplotypes may be more quickly generated for DNA databases and forensic cases may be solved more efficiently.

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O18

High resolution analysis of male genomes by the addition of eight biallelic polymorphisms to the classic 8-STR forensic haplotype

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DNA typing of male specific polymorphisms is a well established procedure of molecular analysis.

A haplotipe of 8 different human male Y-specific STRs (DYS19, DYS389 I and II, DYS390, DYS391, DYS392, DYS393 and DYS385) – selected in 1997 by the seminal work of a group of laboratories (1), has been intensively used for forensic studies and is now applied to real forensic casework. This haplotype has also been effectively used to address specific problems of population genetics (2).

One of the largest archives of populational data is currently maintained for the 8-Y-STR haplotype, that qualifies to become the starting point for future activity on male identity profiles.

We (3) and others (4) have used this haplotype to investigate the genetic variability of a large sample of Italian males.

Following this study, a collection of male genomes typed for the for the 8-Y-STR haplotype has been established at our laboratory for future typing activity.

We have now reinvestigated 50 of these genomes with a battery of eight single nucleotide polymorphisms (SNPs) mapping to the Y-chromosome (M170, M172, M173, M17, TAT, M89, M9 and 92R7) (2,5). Combination of STRs and SNPs has proved as a powefull tool in population genetics studies (6), but its extension to forensic application has not been deeply investigated yet.

The purposes of this study are:
a. refining the identification power of the routine Y haplotype used in our casewok;
b. increase the ability to infer evolutionary relationship between populations by the analysis of male profiles;
c. establishing a quick and relatively inexpensive way to type SNPs

The results of this study show that:
a. the use of genealogically identified groups permits a more appropriate comparison of the italian population with other european groups, making possible inferences on population genetic history and investigation of population structure
b. the forensic efficiency of the extended haplotype is also considerably enhanced
c. the new loci can be effectively typed by a quick protocol involving PCR coamplification, restriction and electrophoretic analysis

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O19

Development of a tetraplex PCR system for the genetic analysis of X-chromosomal STR loci

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We have investigated the four X-chromosomal STR loci HPRTB (Edwards et al., Genomics 12:241, 1992), STRX1 (Edwards et al. Am. J. Hum. Genet. 49:746, 1991), DXS101 (Allen et al., Hum. Mol. Genet. 2:1508, 1993), and DXS8377 (Hu et al., Hum. Genet. 98:178, 1996) to develop a rapid single tube PCR typing system suitable for fluorescent detection. Whereas HPRTB and STRX1 contain tetrameric repeats, DXS101 and DXS8377 have trimeric repeats. All four loci are highly polymorphic having up to 18 common alleles at each locus.

The published primer sequences were slightly modified to achieve more uniform PCR conditions required to perform a tetraplex amplification assay. The four loci were dye-labelled in sets of two primer pairs each to be detected in the following size ranges: FAM – DXS101 (178–232 bp) and HPTRB (263–299 bp); HEX – DXS8377 (204–261 bp) and STRX1 (308–332 bp). PCR was performed using a Perkin Elmer 9600 thermocycler, and separation of the fragments was achieved by capillary electrophoresis using an Applied Biosystems Prism 310.

After optimization of the PCR conditions, allelic ladders were composed from sequenced fragments for each locus for routine genotyping controls. In the STRX1 system, an unusual group of alleles was found that could be characterized by the presence of a single trimeric repeat as well as a 2-bp deletion in the flanking region. Thus the corresponding STR fragments could be described as "12.3, 13.3, 14.3, etc" based on the repeat structure, but as "12.3, 13.1, 14.1 etc." based on the total fragment length variation. It seems that the group of alleles with the incomplete repeat have evolved independently from the other alleles characterized by a regular tetrameric repeat structure.

Finally, we have performed a population study based on 60 family trios (30 male, 30 female offsprings) with confirmed paternity from routine casework to evaluate the forensic efficiency values as well as mutation rates of our X-chromosomal tetraplex PCR system.

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O20

13 Y-chromosomal STRs in a Vietnamese population

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We present the frequency distributions of 13 Y-specific STR polymorphisms (DYS19, DXYS156, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 and YCAII) and the frequency of the combination of these haplotypes in Vietnamese males.

Materials and Methods

DNA was extracted from saliva swabs of 119 unrelated Vietnamese males using the Proteinase K/Chelex method. Amplification of the STRs was carried out in three multiplex reactions: DYS19, DYS389I and II, DYS390, DYS393 (Y-multiplex I), DYS385, DYS437, DYS438, DYS439 (Y-multiplex II), DXYS156, DYS391, DYS392, YCAII and Amelogenin (Y-multiplex III). For the Y-multiplex III some primers were newly designed. PCR fragments were analyzed using ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Results and discussion

In 119 Vietnamese samples, 112 different haplotypes were observed. The 106 haplotypes of them were unique and the others shared by 2 or 3 persons. For an informative core set of 8 Y-linked STRs constantly updated in Europe (DYS19, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393), 104 different haplotypes were detected and further inclusion of YCAII yielded 105 different ones. Diversities of the individual loci ranged from 0.33 (DYS438) to 0.95 (DYS385) and the haplotype diversity was 0.99. For the extremely polymorphic DYS385, the allele 13/18 was most commonly found in 11.7% of Vietnamese samples, whereas the allele 11/14 was common in the Germans (33.2%, data not shown).

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O21

Haplotype frequencies for 13 Y-chromosomal STRs in an Arab population sample from Syria

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Analysis of Y-chromosomal STRs has become increasingly important because of their usefulness in forensic and population genetics. Although many populations have already been analysed, there are only few data on Arabic populations. A total of 13 polymorphic STR loci on the Y- chromosome were analysed in a Syrian population sample of 113 unrelated males (the majority from Lattakia).

DNA was extracted from saliva swabs using the Proteinase K/Chelex method.

The Y-STRs were amplified in three multiplex PCR reactions: DYS 19, DYS 389 I and II, DYS 390, DYS 393 (Y-multiplex I); DYS 385, DYS 437, DYS 438, DYS 439 (Y-multiplex II); DXYS 156, DYS 391, DYS 392, YCA II and amelogenin (Y-multiplex III).

PCR fragments were analyzed by denaturing capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer. The frequencies of compound haplotypes were determined. In 113 Syrian samples, 108 different haplotypes were observed, 104 haplotypes of them were found to be unique and the others were shared by 2 or 3 persons. The haplotype diversity was 99.02%.

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O22

Phylogeny of the mtDNA haplogroup U6: analysis of the sequences observed in North Africa and Iberia

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The haplogroup U6, defined by transitions at np 16172 and 16219 in the hypervariable region I (HVRI) of mitochondrial DNA (mtDNA) is characteristic of North African populations, reaching the highest frequencies (~20%) in Berbers. The cluster U6 has been subdivided into two subgroups, U6a defined additionally by a transition at np 16278 and U6b having a transition at np 16311.

Outside North Africa, the highest frequencies (10–16%) are observed in the Canary Islands, which are located in the Atlantic Ocean close to the northwest African coast, and which were a centre of the Iberian overseas expansion from the fifteenth century. There is indeed a typical Canarian U6 sub-haplogroup, U6b1, defined by an additional substitution at np 16163, which encompasses 93% of the U6 sequences observed in the Canary Islands, pointing to a unique introduction of North African lineages into these islands around 2,800 ± 900 years ago, with the less frequent sequences likely to have been introduced subsequently.

In Iberia, 14 U6 sequences have also been reported, a much higher frequency than in the rest of Europe (only 2 in a European-wide database). Out of 14 U6 sequences, 11 were from the north of Iberia: 7 in North Portugal (7.0%); 2 in Galicia (2.2%); and 2 in northeast Spain (1.7%), the remaining 3 being described in a general sample from Portugal (5.5%).

In order to clarify whether the Iberian U6 sequences could have been introduced in one major prehistoric event, as seems to have been the case in the Canary Islands, or whether they are the outcome of more recent introductions, e.g., during the Islamic period, we took a closer look at the different HVRI sequences. Of the Iberian U6 sequences, 2 belong to the Canarian specific sub-haplogroup U6b1, and these were likely to have been introduced from these islands (a short-lived Guanche slave trade is recorded). The remaining 12 are very diverse, corresponding to 9 different haplotypes, 5 of which belong to U6a and 4 to U6b. Only 4 sequences share the same widespread U6a substitution pattern 16172–16189–16219–16278. Taken together, this does not support the hypothesis of a single founder for this haplogroup in Iberia. Whether it was introduced in a single event from a source population with a diverse set of U6 lineages or whether it was introduced over a long period from several sources is not clear. Since it is currently not possible to infer the U6 founder types, it is impossible to date its introduction. An enlarged North African database would aid in the solution of this puzzle.

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O23

A novel mt-DNA coding and D-loop analysis for forensic identification based on pyrosequencing

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The use of PCR based analysis has made it possible to perform forensic DNA analysis on minute amounts of DNA found at the scene of a crime. Such evidence materials are saliva stains, shed hairs, teeth, bits of bone, skin cells and fingerprints on various items. Sequencing of mitochondrial DNA will overcome the limitation of low DNA copy number due to the high copy number per cell. In routine analysis today is the two most hypervariable regions, HVI and HVII of the control region (the D-loop) sequenced. A major drawback using complete sequencing of the mitochondrial D-loop is that it is a rather time-consuming analysis. We have development a pyrosequencing based system to simplify and optimise routine forensic mtDNA analysis.

Pyrosequencing, a new method first developed for detection of known single-base changes, is gaining widespread use in many areas. Further developments of pyrosequencing allow short stretches of DNA sequence (30–50 bp) to be determined easily. In comparison to many other techniques for SNP analysis is the analysis time reduced substantially when using pyrosequencing. A parallel analysis of 96 samples is done in less than 30 minutes. The method is based on a rapid, non-electrophoretic, single-tube sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light. The light, proportional to incorporated nucleotides and released pyrophosphate (PPi) is detected in real-time using a luminometric detection system.

This pyrosequencing-based system for human identification is based on analysis of 10 PCR fragments covering highly informative sites throughout the entire mitochondrial genome. The system is based on analysis of a total of16 pyrosequencing reactions, 4 HVI, 4 HVII and 8 coding region reactions using the PSQTM 96 instrument (PyroSequencing AB). An evaluation using 200 previously mtDNA sequenced samples from blood donors as well as a number of previously sequenced forensic evidence materials has been done. The system has further been tested with high success rate on a number of recent forensic cases. This forensic analysis system enabling analysis of the D-loop as well as parts of the coding region of the mtDNA has proven to be both extremely sensitive, very rapid, reliable and have a higher discrimination power.

The request for mitochondrial DNA analysis has increased notably during the past years and has been very useful in cases with circumstantial evidences. This fast system to obtain mtDNA sequence, pyrosequencing, will furthermore enable use of mtDNA analysis in a case during the first very important days of the criminal investigation. In addition, the system will be substantially more informative than d-loop sequence analysis since it is based on analysis of polymorphic sites in the coding region in addition to the D-loop region.

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O24

Mitochondrial DNA (mtDNA) variation in Indian populations

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Indian population is genetically, geographically and socio-culturally very diverse. The patterns of present day genetic diversity in India offer important clues to the evolutionary history of human populations. Caste structure has played an important role in shaping up the observed genetic variation. Therefore it is challenging to analyse and document patterns of genetic diversity. We have surveyed mtDNA sequence variation among 600 individuals from different caste and tribal populations using PCR amplification and sequencing of HVR1 region. Estimates of mtDNA sequence diversity ranged from 0.004 to 0.028 and GST estimates among castes groups were lower than tribal populations. Comparisons are made with mtDNA haplotypes from Caucasian, Asian, African and other Indian populations to assess relative position of these populations and the forensic usage of mitochondrial diversity in Indian populations.

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O25

Investigation of chimerism in a healthy, adult female by means of minisatellite and microsatellite typing

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Introduction

Chimeric individuals are characterized by having cells with different genetic patterns originating from two or more zygotes. Within permanent chimeric individuals "blood chimeras" or "twin chimeras", which result from in-utero junctions of twins and "whole body chimeras", where the coexistence of the different cell lines is not restricted to hematologic cells but spread over various tissues, can be distinguished.

Whole body chimerism (tetragametic or dispermic chimerism) is characterized by double parental or double paternal contribution of markers. In very rare cases double maternal contribution has also been detected.

We investigated blood and tissue samples of a healthy female, showing irregular agglutinations in serological blood grouping, in order to verify the chimeric status and clarify its mode of origin.

Materials and Methods

EDTA whole blood samples were withdrawn from the proposita and her parents. Additionally, buccal swabs and finger nails were collected from the proposita. DNA extraction was either performed by Chelex® extraction, the "salting out method" or Qiagen DNeasy Tissue kit®. STR typing was conducted employing the AmpFISTR SGM Plus and Profiler Plus kits® as well as the Powerplex™ 16 kit, the genRes HumACTBP2 (SE33)kit® and an in-house method for D12S391. Subsequent minisatellite typing had to be conducted using non-commercially available methods for YNZ22, D1S80 and ApoB.

Results and discussion

STR typing with AmpFISTR SGM Plus and Profiler Plus kits® (13 different STR loci and Amelogenin) revealed the presence of two maternal and one paternal allele at 8 loci in blood and tissue samples of the proposita. In some loci mixed patterns could not be observed due to shared alleles between the parents, whereas in 2 loci no double maternal contribution was observed despite maternal heterozygosity. Amelogenin gave no indication for the presence of a Y-chromosome.

Thus twin chimerism could be ruled out, but the rare case of double maternal contribution had to be assumed. Further investigations including 6 more loci using Powerplex™ 16, the genRES HumACTBP2 kit and D12S391 finally revealed the presence of two paternal alleles at Penta E locus (Powerplex™ 16). VNTR typing also clearly confirmed that the chimerism of our proposita is a result of double parental contribution of alleles.

Conclusion

In an age of highly discriminating STR assays, the origin of a chimeric individual can easily be clarified. In some cases, the use of minisatellite systems is helpful.

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O26

Mutations and Aneuploidies: Their prevalence and impact on forensic casework

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The Forensic Science Service (England and Wales) has compiled data from two large scale systematic surveys designed to assess the incidence and types of mutations occurring at ten STR loci commonly used in forensic casework in the UK. The genetic anomalies investigated included germ-line mutations, somatic mutations, null (primer binding site) mutations and other chromosomal anomalies. In this paper we report the results of these surveys.

The first survey was a screen of approximately 600,000 STR profiles held on the UK National DNA database and was designed to identify profiles exhibiting anomalous band patterns. Over 500 such profiles were identified and studied. Clear trends in the patterns emerged and these will be presented together with representative examples.

The second survey involved a study of approximately 50,000 parent-child allele transfers. These were collected during routine familial testing in civil cases of disputed parentage in the Forensic Science Service Paternity Analysis Unit. Approximately 60 putative mutations were identified as inconsistencies with Mendelian Inheritance patterns and these have been catalogued. The relatively large size of the dataset enabled a detailed evaluation of trends. This has provided insights into the prevalence and types of genetic rearrangements that are likely to be encountered during familial testing.

The impact of these findings and the implications for using STR loci in forensic casework and familial testing will be considered.

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027

Mutation rates at twenty-three different short tandem repeat loci

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In a collaborative study we calculated mutation rates at 23 different STR loci: CD4, CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1132, D8S1179, D12S391, D13S317, D16S539, D17S976, D18S51, D19S433, D21S11, F13A1, F13B, FES, FGA, SE33, TH01, TPOX and VWA after investigation of nearly 24.000 meioses.

The alleles taking part in mutations were sequenced. All mutations were found to be either gains or losses of complete repeats. The mutation rates at the individual STR loci are given in the following table:

Locus	Number of meioses	Number of mutations	Mutation rate x 10^{-3}	95% confidence limits
CD4	1091	0	<0.9	0 – 3.4
CSF1PO	271	0	<3.7	0 – 13.6
D2S1338	404	1	2.5	0.1 – 13.8
D3S1358	1041	0	<1.0	0 – 3.5
D5S818	795	0	<1.3	0 – 4.6
D7S820	795	0	<1.3	0 – 4.6
D8S1132	121	1	8.3	0.2 – 46.0
D8S1179	989	1	1.0	0 – 5.6
D12S391	433	0	<2.3	0 – 8.5
D13S317	795	1	<1.3	0 – 7.0
D16S539	504	0	<2.0	0 – 7.3
D17S976	611	0	<1.6	0 – 6.0
D18S51	989	1	1.0	0 – 5.6
D19S433	404	0	2.5	0 – 9.1
D21S11	1038	3	2.9	0.6 - 8.4
F13A1	382	0	<2.6	0 – 9.7
F13B	1374	0	<0.7	0 – 2.7
FES	1656	1	0.6	0 – 3.4
FGA	2055	6	2.9	1.1 – 6.4
SE33	1733	9	5.2	2.4 – 9.9
TH01	2735	0	<0.4	0 – 1.3
TPOX	271	0	<3.7	0 – 13.6
VWA	3164	7	<2.2	0.9 – 4.6
Total	**23651**	**31**	**1.3**	**0.9 – 1.9**

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STR-typing in a pair of chimeric twins

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Introduction

In man congenital chimerism is due to the coexistence of two genetically different cell lines either in the whole body or limited to the blood. Blood stem cells can migrate through anastomoses of intrauterine blood vessels during pregnancy of twins and produce blood cells in the other embryo for a lifetime. Therefore these individuals are called "twin chimeras" which differ from "dispermic chimeras", who derive from the fertilization of one or two maternal nuclei by two sperms and their growth into one body.
A pair of chimeric twins is well known to our laboratory and was intensively examined with serological, biochemical and cytogenetical methods two decades ago (Pausch et al. 1979). Further examination of three dispermic chimeras proved the benefit of mini- and microsatellite typing in these fascinating cases. Therefore we decided to perform STR-typing on these twin brothers. We expected to see a mixture of the genetic pattern of the two brothers in the blood. In the solid tissue we expected only the genetic pattern of the individual brother himself, which was inherited to him from his parents, not what he was supposed to have got "in exchange" from his twin brother during pregnancy.

Materials and Methods

Blood samples and buccal swabs were taken from the twins and examined with the AmpFlSTR SGM Plus Kit (Applied Biosystems) on an ABI Prism 310 capillary electrophoresis instrument (Applied Biosystems).

Results and Discussion

STR-typing revealed the coexistence of three different alleles at 5 out of 11 loci in the blood samples, which showed different signal intensities in capillary electrophoresis. The samples taken from inside their cheeks showed the same phenomenon in one of the twins and could not clearly be excluded in the other one. All alleles found in the blood or the buccal swab of each brother have been found in the blood of his twin brother. Interpretation of the STR-typing results was difficult due to the apparently low proportion of the third allele in the blood and probably the buccal swab of one brother. Additionally, in all but two cases the third allele was found in "stutter position", which is a complete tetrameric repeat shorter than the major allele.

Conclusions

As additional alleles present in the other twin brother have not been expected in the buccal swabs of the twins, the examinations have been extended on other tissues, which are not supposed to be potentially contaminated with leucocytes, and further genetic markers.

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029

Identification of a phenotypically normal tetragametic chimeric fertile woman by HLA and STR typing

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We studied an unusual tetragametic phenotypically normal and fertile 46,XX Caucasian female, who was identified because her blood HLA typing showed what appeared at first to be maternal exclusion of two of her three children. These results were confirmed by DNA analysis of 18 STRs (Short Tandem Repeats). The propositus, her mother, two brothers and three children were subsequently studied by advanced HLA molecular typing and by DNA of 18 different STRs. The propositus's blood showed the presence of only two HLA haplotypes and only one or two alleles for each STR (homozygous or heterozygous). Yet, other tissues including hair follicles, buccal epithelium, skin fibroblasts, thyroid, and fibroblasts and epithelial cells from urinary bladder, showed evidence of four HLA haplotypes. In some of the tissues, two haplotypes were found as preponderant, while the reverse was found in the other tissues. Such chimerism was confirmed using several autosomal STRs. Extensive cytogenetics analyses showed a 46 XX chromosome constitution in blood and fibroblasts. This finding was corroborated by the absence of Y-specific DNA signal by amelogenin typing in the propositus samples analyzed.

Mixed lymphocyte culture and cell mediated lysis demonstrated lack of reactivity of allogeneic cell proliferation and cytotoxicity in the presence of HLA haplotypes absent in the blood but present in the other tissues of the propositus. This resulted in immunologic tolerance to four different HLA haplotypes in this tetragametic individual, suggesting the possibility of finding immune tolerance to bone marrow transplantation in individuals with this type of chimerism using donors from close relatives without matching for blood HLA haplotypes. Furthermore, because two of the propositus's children received one HLA haplotype from the father and the second haplotype was not found in the blood of the propositus, it is possible to find cases that mimic parental exclusion unless one studies more than one tissue of the person under scrutiny.

Or results are best explained by a chimerism in several tissues of the body, other than blood, which resulted from two maternal gametes, fertilized by two paternal spermatozoids forming a two cell zygote, with distinct genetic constitution, that differentially populated the body.

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030

Megaplex analysis of a Mongolian population from the Egyin Gol site (300 B.C.–200 A.D.)

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Genetic analyses of archaelogical remains may provide information of considerable importance to the understanding of human past. In order to investigate the history of Mongolian populations (e.g. social organization, migrations…), a collaborative study has been set up between the Department of Anthropobiology of the Paul Sabatier University (Toulouse, France) and the Department of Molecular Anthropology of the Institute of Legal Medicine (Strasbourg, France). In the framework of this study, we analyzed the skeletal remains of 54 specimens excavated from the Egyin Gol site, a necropolis located near the Egyin Gol river in northern Mongolia. The burial site is linked to the *Hunnu* period and extends from the third century B.C. to the second century A.D. Little is known about the history of this nomad population. The geologic conditions at this site were cool and dry, and DNA preservation was exceptional allowing nuclear markers such as microsatellites to be studied.

Molecular analyses were performed on long cortical bone samples with the AmplFlSTR Profiler Plus kit (Applied Biosystems) which allows the amplification of 9 STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D13S317, D7S820) and the amelogenin locus.

Amplifications were sucessfull for 45 of the 54 specimens analysed: four samples failed to yield any detectable human nuclear DNA in several independent extracts whereas five contained too few template molecules to provide reproducible results. In samples where only some of the STR loci could be amplified, amplification failure was usually observed with the larger STRs which is consistent with ancient human remains DNA analysis. Molecular sexing confirmed archeological data based on morphometric parameters and allowed to determine the gender of four juvenile skeletons. STR analysis showed close relationships between several specimens (e.g. siblings, parents…). Genetic homogeneity among the population studied was observed through statistic analysis using the Genetix software. The observed allele frequencies were determined and compared to those of Caucasian and Asian populations.

The present study should facilitate the interpretation of the burial site since morphological indicators of kinship are much less precise than the genetic data obtained by analysis of nuclear DNA. Moreover, we demonstrate for the first time that it is possible to obtain multiplex STR database of a past population, allowing comparison with present populations.

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031

Multiplex-PCR of short amplicons for mtDNA sequencing from ancient DNA

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Amplification of each hypervariable region of the mtDNA Control Region in two separate PCR reactions (>400 bp) or using primers to amplify two overlapping segments of each region (›200 bp) are the strategies most employed in forensic mtDNA typing. However, successful mtDNA analysis from extremely old and/or highly degraded or damaged DNA samples sometimes requires amplification of very short amplicons. We here describe a multiplex-PCR method to generate six overlapping short amplicons (100–130 bp) in two separate PCR reactions of non-overlapping fragments for full sequencing of the whole hypervariable region I (HV1). The performance of this multiplex system has been evaluated not only on ancient bone remains (5000–4000 BP) but also on different forensic samples with highly degraded DNA (bone remains, hair shafts, fixed samples…) that yielded negative PCR results with the mtDNA amplification strategies usually employed in forensic genetics. The multiplex-PCR methodology described in this study also illustrates a potential throughput strategy to reconstruct the sequence of both coding and non-coding regions of the mtDNA genome from ancient DNA.

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DNA identification of World War II soldiers from skeletal remains by using mtDNA SSO hybridisation assay and mtDNA sequencing

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Skeletal remains of Finnish soldiers deceased in World War II and buried in the battlefields in Karelia, Russia for 50 years were recovered and investigated in an attempt to identify them. The primary identification was based on war records and documentation of personal effects. This was further confirmed with comparison of mtDNA with assumed maternal relative. DNA extraction from powdered bone material was performed using standard phenol-chloroform protocol and purified after Centricon concentration with QiaQuick-purification kit (QIAGEN). The primary DNA screening of individuals (skeletal remains) was achieved by using a sequence specific oligonucleotide (SSO) hybridisation assay. Fifteen probes specific for the second hypervariable region (HVRII) of the mtDNA control region were used to type the bone sample and the blood sample donated by maternally related family member of the supposed soldier. In cases where SSO hybridisation assay could not resolve multiple individuals in a single grave sequencing of mtDNA control region (HVRI and HVRII) was added to the project protocol. PCR was carried out according to FBI laboratory mtDNA sequencing manual for both HVRI and HVRII regions and sequencing reactions were performed with BigDye Sequencing kit (Applied Biosystems). Altogether 105 samples were succesfully amplified and analysed. The autenticity of sample DNA was controlled with positive and negative control samples in each step of extraction, amplification and sequencing. The best results were obtained from femur and tibia indicating that DNA is well preserved in compact bone material even after over 50 years in acidic soil of Karelian forest area. The sequence comparison was performed using SeqEd 1.0.3 software and the autenticity of results was controlled with comparison to Anderson sequence of mtDNA control region. The obtained sequences were also compared to our Finnish mtDNA sequence data (n=120) in order to calculate the occurence of particular mtDNA sequence in the database.

To conclude, our data shows that mtDNA can be recovered from human bone material buried over 50 years in boreal soil. Furthermore, mtDNA sequence analysis from old human remains appears promising for forensic human identification.

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The Combined DNA Index System

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Databanks, that contain DNA profiles of convicted felons, missing persons, and/or profiles from evidence from cases, are useful for providing investigative leads for resolving certain crimes. The FBI Laboratory has developed the COmbined DNA Index System (CODIS) to assist in resolving crimes, particularly violent crimes, and prevent further crimes by quickly identifying the perpetrator. CODIS enables federal, state, and local crime laboratories to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders.

CODIS is implemented as a distributed database with three hierarchical levels – local, state and federal. NDIS (the National DNA Index System) is the highest level in the CODIS hierarchy and enables laboratories participating in CODIS to exchange and compare DNA profiles on a national level. All DNA profiles are generated at the local level (LDIS) and then flow to the state (SDIS) and national levels. SDIS allows laboratories within a state to exchange DNA profile information. The tiered approach allows state and local agencies to operate their respective databases according to specific legislative or legal requirements.

DNA records from a number of sources can be obtained, stored, and compared in CODIS. The sources are: 1) Convicted Felons – which are persons convicted of crimes, defined under state statutes; 2) Missing persons and their close biological relatives, which are persons reported missing, and sought, and their biological relatives, such as parents, siblings and children; and 3) Forensic evidence. Lastly, there is a population allele frequency database index for statistical applications.

In order for DNA profile databanks to be useful at a national level, standardization of the genetic markers used across laboratories is essential. To take full advantage of the power of STR typing and to ensure comparability of DNA profiles, the following 13 STR loci have been selected as consensus core markers for CODIS: CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. To achieve high levels of discrimination, laboratories in the United States routinely type all 13 core STR loci when profiling convicted offenders, and the typing of the same loci is attempted when analyzing casework.

In addition to STR loci, determining the sequence of a portion of the mtDNA molecule extracted from hair shaft, bones, or teeth can assist in resolving a number of crimes, especially for identifying remains of missing persons. The central function of the software is to facilitate searching of a mtDNA nucleotide sequence developed from an evidentiary sample against one or more sequence databases. Two types of searching are enabled: 1) comparison of a single profile against the profiles in the Index; and 2) a pairwise search in which every profile is compared to every other profile in the selected population database(s). The implemented CODIS missing persons database and mtDNA profile searching software are known as CODIS^{MT}. To support case interpretation a mtDNA Population Database is available and will be periodically posted on the Internet *(Forensic Science Communications* at http://www.fbi.gov). The database contains SWGDAM mtDNA population data (currently ›4000 samples) that have been sequenced for both hypervariable regions I and II. Extant SWGDAM population data are comprised of all major racial groups.

Other countries may obtain the CODIS software, and the software will be provided free of charge to appropriate authorized agencies. Currently CODIS is installed in more than 131 laboratories in the U.S. and 34 laboratories in 12 other countries. NDIS currently contains more than 520,000 profiles from 34 states, the U.S. Army Crime Laboratory and the FBI.

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The National DNA Data Bank of Canada – A Laboratory Bench Retrospective on the First Year of Operation

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In June 2000 the National DNA Data Bank of Canada began operations to process biological samples from criminal offenders charged with serious offences. The success of the data bank as an investigative tool as measured by the number of cold hits recorded in its first year of operation (16), reflects the developmental systematic approach elaborated during the 18 months preceeding its initiation. Several issues were addressed during this initial phase: 1) the collection of samples, the commercial development of collection kits and the training of law officers responsible for obtaining biological samples from offenders, 2) the development and optimization of extraction and purification procedures for robotic workstations, 3) the scaling up of sample throughput at analytical workstations, 4) the development and implementation of a training strategy for analysts, and 5) the development of a specialized software suite to manage sample flow. Developmental strategies were predicated on an anticipated 30,000 to 97,000 sample submissions to the data bank on a yearly basis, requiring, in turn, for the overall process to accommodate anywhere between 60,000 to 194,000 amplification reactions to be carried out with both the AmpFlSTR® Profiler Plus™ and AmpFlSTR® COfiler™ megaplexes on each sample (blood, buccal swabs or hair samples; all on FTA cards) received. The ultimate objective was to automate the entire DNA typing process in order to meet the highest standards of quality control, efficiency and reduced operation costs. Specific goals were: 1) to have a tight control over sample traffic, 2) to allow for batch processing of samples while maintaining the capability to customize processing conditions for each sample, 3) to allow for sample re-processing at any step in the analytical process, and 4) to maintain flexibility during the development and optimization of analytical procedures for the robotic workstations. In the end, a fully integrated and automated approach requiring very little human intervention was successfully developed. This platform represents the cornerstone of the national data bank. With two pre-PCR and two post-PCR robots, a group of six analysts can handle a yearly production of up to 120,000 PCR assays. This approach makes the most efficient use of samples, reagents, personnel time and robotic workstations, while ensuring that all samples are processed error-free in the shortest possible time. This approach also fulfills the special requirement to separate the criminal offender's personal information from the genetic information to safeguard genetic privacy.

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O35

Four unusual cases of disputed paternity evaluated by a likelihood ratio test based on the number of alleles shared identical by state (IBS)

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Case 1. Individual C claims that individual S is his father. S says that the true father of C may be his own father. Neither the mother of C, nor other relatives of C or S are available for analysis. Then, we want to evaluate the following two probabilities, X and Y, and obtain the likelihood ratio (LR) X/Y (G stands for genotype):

X) Pr(GC | G_S, S is father of C)
Y) Pr(GC | G_S, S is half sib of C)

Case 2. Individual C pretends to be half sib of individual S through a common deceased father. S denies any relationship with S. Both the mothers of C and S (MC and MS , respectively) are available for analysis. Then, we want to evaluate the the following two probabilities and the corresponding LR:

X) Pr(GC | G_S, G_{Mc}, G_{Ms}, S is half-sister of C)
Y) Pr(GC | G_S, G_{Mc}, G_{Ms}, S is unrelated to C)

Case 3. Two subjects, R and S, are daughter of the same mother M. The dispute concerns if they are full sisters or half sisters. Then, we want to evaluate the following two probabilities and the corresponding LR:

X) Pr(GR , G_S | G_M, R and S are full-sisters)
Y) Pr(GR , G_S | G_M, R and S are half-sisters)

Case 4. Individual C claims to be son of S, whose DNA has been extracted from bones. However, the doubt is raised that the true father of C is a nephew of S. Then, we want to evaluate the following two probabilities and the corresponding LR:

X) Pr(G_C | G_S, S is father of C)
Y) Pr(G_C | G_S, S is granduncle [grandfather's sib] of C)

We first applied the usual method to compute the LRs of the various alternative hypotheses. This required evaluating the conditional probabilities of observing the disputed genotype(s), given the undisputed genotypes and the frequencies of the observed alleles. Using this method, different formulas must be applied within each hypothesis, depending on the configurations of the shared alleles.

Then, we applied a different approach, based on the probabilities z_0, z_1, and z_2 that two subjects with a given relationship share 0, 1, or 2 alleles identical by state (IBS) at a number of loci. This method is conceptually and computationally easier and more robust than the usual method. A unique set of z_0, z_1, and z_2 values characterizes each relationship, and their values depend on the heterozygosity of the locus (H) rather than on the frequencies of particular alleles. For example, the mathematical relation between H and z_0, z_1, and z_2 is linear In the case of parent-child pairs (z_0 is 0, and z_1, and z_2 equal H and 1-H, respectively). For other relationships the functional dependence of z_0, z_1, and z_2 on H is more complex, and we obtained their values by computer simulations.

We show an analysis of the statistical power of the two approaches. As the IBS method is based on pairs of relatives rather then on multiple individuals, we investigated the practical relevance of typing additional undisputed relatives, when available, in increasing a given LR.

036

On the Statistical Analysis of DNA Mixture Evidence

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The statistical treatment of DNA evidence in crime cases, and the subsequent presentation of the obtained evidential strength in court, is by now reasonably well established. The common method is to present likelihood ratios (LRs), comparing the prosecution and the defense hypotheses concerning the circumstances of the crime, see Evett and Weir (1997). A standard case with mixture evidence involves DNA profiles obtained from, respectively, a mixture stain, usually consisting of blood or semen, found at the scene of the crime, as well as personal samples from typically a victim and a defendant. The LR is formed from likelihoods calculated assuming hypotheses like

- H_p: The victim and the defendant contributed to the stain
- H_d: The victim and an unknown, unrelated person contributed to the stain.

The evaluation involves various standard assumptions, concerning both genetical and statistical aspects.

Situations arise, however, where the described approach becomes less convenient. In particular, this is true when more than two relevant hypotheses are proposed in a case. For instance, the number of contributors to a stain might not be known, hence, it would be appropriate to consider several possible scenarios. A general Bayesian framework is suitable for handling such cases in the sense that an arbitrary number of hypotheses can be included in the analysis. In addition, this approach enables the inclusion of other relevant data via a prior distribution. The approach coincides with the classical LR-approach for special cases, however, the evidential strength is now presented by means of a posterior probability distribution, characterizing the set of hypotheses. The presence of other complicating factors, like family relations between donors, unavailability of personal sample from involved person, and uncertainty in the obtained profiles, can also be handled within the proposed framework. We report from our efforts to assess the evidential strength in such cases, and demonstrate the coherence of the proposed approach.

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037

Old family secrets exposed

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An elderly man and woman died after being burned in an automobile accident. DNA typing in 13 autosomal STR loci strongly confirmed that they were brother and sister. But further typing, on four additional presumed siblings, revealed some surprising and instructive genetic patterns. The additional information contradicts the original conclusion, and also reveals a remarkably precise (and lurid) story of events many years ago.

The investigation was guided by several precepts that have general application. Even four alleles among three children can be enough to guarantee three parents among them, if one allele occurs three times. Such a pattern in this case indicated partitioning the children into two sets of possible full siblings. Nonetheless, a half-sibling hypothesis between the two sets was not sufficient to explain the small amount of genetic variability represented among them. Various possibilities of incest or inbreeding are thus under suspicion. For example, it is suggestive of father-daughter incest if a set of full siblings exhibits three or fewer alleles per locus in each of many polymorphic loci. Observations of these sorts, combined with likelihood ratio calculations as a final step to choose among various possibilities, thus can lead to a plausible understanding of even a tangled set of family relationships.

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038

Mutations, and the probabilistic approach to incompatible paternity tests

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Paternity tests are currently carried out by typing a set of microsatellite loci (STRs). This class of polymorphisms is known to be prone to germline mutations. The gametic mutability of those STR loci that are most often used for paternity tests has been closely examined in studies (Brinkmann et al 1998: Henke and Henke 1999), which have estimated overall mutation rates per locus, μ, from a considerable volume of segregations. On the basis of these studies there is now general consensus in defining mutations as non-sporadic events in human lineages.
In the evidentiary context of a typical forensic test, mutations may lead to apparent paternal/parental exclusions. This poses problems in the interpretation of the underlying biological evidence, and the soundness of some *ad hoc* rules traditionally used to manage incompatible tests (such as: "non-fatherhood is certain after the occurrence of two/more than two exclusions") is not guaranteed.
We have recently shown how a probabilistic analysis can be applied to incompatible genetic evidence in paternity tests, leading to its quantification in terms of a likelihood ratio, LR, for paternity vs. non-paternity. Our approach incorporates the following ingredients:

a. "Incompatible" genotype triplets explicable by assuming a single mutation are considered.
b. Arbitrary, possibly different, mutation rates μ in paternal and maternal germlines are allowed.
c. For each locus, allele-specific mutation transition rates (i.e. for allele x to mutate to allele y) are derived in terms of μ, based on reasonable biological assumptions including stepwise mutation and stationarity of gene frequencies (Dawid et al. 2001b).
d. For each locus showing incompatibility, the probability (given the parental genotypes) of obtaining the child's genotype under the hypothesis of paternity (assuming a single mutation), and the corresponding probability under the alternative non-paternity hypothesis (assuming no mutation), are computed, and combined to form a likelihood ratio.
e. The overall likelihood ratio is obtained by multiplying together these LRs for incompatible segregations with the LRs from compatible loci.

Our proposed mutation model is compared with other mutation models suggested in the literature, and sensitivity analysis is performed on model parameters. We also show how to compute upper bounds on the LR in the very general case when one does not want to adopt any particular mutation model.
Our results, appropriately modified, could be used to obtain estimates or bounds on LRs allowing for other sources of false exclusion (e.g. laboratory errors or falsifications), so pointing the way towards a general computational solution to the problem of uncertain paternity.

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039

Assessing relationships in an ancient skeletal collection by the number of alleles shared identical by state (IBS) among pairs of individuals

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In previous studies, we described a collection of skeletons from a 18th century burial ground at Goslar, Germany, and showed genotype results of 26 individuals typed for up to 9 STRs with the Amplifier© kit, plus amelogenin. No significant differences between allele and genotype frequency distribution were found in our sample compared with present-day German population; however, the level of allele sharing among some of the skeletons suggested that the sample could include some close relatives. In the present work, we reworked at the genotype database and addressed the problem of inferring the relationship between pairs of individuals using an approach based on the probabilities z_0, z_1, and z_2 of sharing 0, 1 or 2 alleles identical by state (IBS) at a number of loci. This method is conceptually and computationally easier and more robust than the usual method based on the population frequencies of the observed alleles.

We selected from the database the individuals with at least 5 successfully typed markers (22 out of 26 examined individuals). Seven samples (30%) had all 9 genotypes, and other eleven (50%) had 7 or 8 genotypes. To infer their possible relationships, we first worked out the probabilities z_0, z_1, and z_2 for each of the 9 loci, and for each of the following five relationships: 1) Non-relatives (NR); 2) First Cousins (FC); 3) Second degree: grandparent/grandson, uncle-aunt/niece-nephew, half sibs, collectively called HS; 4) Full sibs (FS); 5) Parent/child (PC). We simulated 100,000 pairs for each relationship, and obtained the z_0, z_1, and z_2 values as the proportions of pairs sharing 0, 1, or 2 alleles. Simulations were based on the weighted mean allele frequencies in three present-day German-speaking populations. We then paired each individual of our sample with all others, and determined the number of loci they had in common and the number of shared alleles (0, 1 or 2) at each locus. In each pair, we substituted the value of the observed number of shared alleles at each locus with the corresponding estimated probability for each of the five relationships. Probabilities were then multiplied, for each pair, across the common loci, separately for each relationship. Thus, the final value was the probability of observing that particular configuration of shared alleles in two individuals, given each specified relationship.

For each pair, we computed four likelihood ratios (LR), using the PC, FS, HS and FC probabilities in the numerator, respectively, and the NR probability in the denominator. Three pairs gave overwhelming evidence that the involved subjects were first-degree relatives. The LRs were 10,468 for pair 21-24 being FS, 490 for pair 3–23 being FS, and 458 for pair 19–24 being PC, respectively. Their nominal P values were higher than 99.7%, and remained significant above the 95% level even after correcting for the multiple comparisons. Noting that individual 24 was a member of two of these pairs, we looked at the LRs of pair 19–21. This pair also ranked high for a LR suggestive of biological relationship. Its maximum LR was 7.9, for the HS relationship (second-degree). Thus, the pedigree of maximum likelihood for this trio of skeletons implies that 21 and 24 are brothers and 19 is a child of 24. Other pairs showed LR values suggestive of relatedness, but the P values were not significant after correction, and we did not accept them.

In conclusion, we identified with high confidence a pair of siblings and a trio of two brothers with a child in our sample of 22 skeletons. This work shows that with a relatively low number of typed loci and a simple method based on IBS, it is possible to approach the problem of assessing relationships among samples of unknown relatedness.

O40

Polymerase slippage in relation to the uniformity of tetrameric repeat stretches

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PCR amplicons of short tandem repeat (STR) loci are often characterized by minor fragments one repeat unit shorter than the product of the main allele. It is widely accepted that polymerase slippage is responsible for the generation of these "stutter bands". STR alleles vary in the extent of stutter artifacts and it is hypotesized that polymerase slippage correlates to the length of repeat stretches consisting of uniform repeats. To test this hypothesis we investigated different length variants for two new STR loci: D7S1517 and D3S1545. Both are small size STRs characterized by PCR fragment lengths less than 160 bp. For D7S1517 we could differentiate 12 alleles (heterozygosity rate: 83%), while 7 alleles could be typed for D3S1545 (heterozygosity rate: 68%). The allele nomenclature of both STRs was established after sequencing of selected alleles showing compound repeat arrays of various numbers of GAAA and CAAA repeats for D7S1517 and uniform (GAAA)n stretches for D3S1545. The D7S1517 system showed a highly variable distribution of the GAAA and CAAA motifs in the repeat array resulting in a considerable variation of the length of the longest homogeneous repeat stretch for alleles of the same overall length. Comparing the length of the longest homogeneous repeat stretch to the degree of stutter generation a strong positive correlation between polymerase slippage and the number of repeats in stretches of uniform motifs was found, but no correlation to the overall number of repeats in compound repeat stretches.

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O41

Evaluation of the STR Typing Kit PowerPlex16 with respect to Technical Performance and Population Genetics: A Multicenter Study

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The recently distributed STR typing kit PowerPlex16™ (Promega, Madison) was evaluated in 19 different European laboratories. The kit amplifies the loci D3S1358, THo1, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, Amelogenin, vWA, D8S1179, TPOX, and FGA. The evaluation focused on the following technical aspects:

- impact of the amount of target DNA,
- balance of loci,
- peak height differences in heterozygotes,
- comparison of genotyping results obtained with primers from another manufacturer,
- weak amplification of a sister allele.

In addition, population genetic data were collected with particular emphasis on allele frequencies and mutation rates for the loci Penta D and Penta E. Each laboratory investigated 150–400 individuals. The data also were inspected for population substructure.

This study shows that the PowerPlex16™ kit is very well suited for data basing. The kit is also well suited for one of the different analysis methods in paternity testing, if the laboratories comply with professional standards and precautions. The power to exclude non-fathers from paternity is high (1 in 500000), while the overall mutation rate is about 3%. When typing results were compared with those obtained by different primers, some inconsistent typing results could be observed (e.g. allelic drop-out and weak amplification of a sister allele). An estimation of the frequency of these inconsistencies will be given.

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O42

Efficancy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci

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STR analysis by multiplex PCR has enabled the forensic scientist to derive DNA profiles from minute amounts of biological sample. Using the commercially available SGM plus kit reliable typing results can be obtained. The PCR reaction conditions for this multiplex system are consistent and robust under the conditions that are outlined in the manufacturers instructions. A complete and accurate STR profile can be obtained from 100 picograms of DNA template. This amount of DNA is contained in the nuclei of no more than 18 diploid cells. By further increasing the sensitivity of the typing system DNA profiling from minute traces of biological evidence such as residues left in a fingerprint or a palm mark comes within reach. Several alternative methods have been described to increase the sensitivity of PCR amplification. Most methods use a preamplification step before locus specific amplification. Another way to increase the sensitivity of PCR amplification is by simply extending the number of PCR cycles. By its simplicity, this method is preferred to preamplification. In this study, we have evaluated the efficacy and the validity of the SGM plus test using an amplification regime of 34 cycles. We obtained valid DNA-typing results from pristine extracts with low DNA-content. Analyses of heterozygote balance, between loci balance and stutter proportion shows that the 34 cycles PCR has its own characteristics.

LCN DNA samples from extracts of bone and teeth that failed to demonstrate typing results using the standard protocol of 28 cycles showed complete SGM plus profiles after 34 cycle amplification.

Aspects of single cell PCR typing were also evaluated. In these experiments, the allele dropout phenomenon was clearly observed.

We have subsequently studied the characteristics and validity of the SGM plus multiplex PCR kit under the 34 cycles regime by analysing LCN samples from a number of simulated and authentic forensic samples. Exhaustive validation studies are in progress in order to procure full accreditation for the LCN DNA-typing technology in our laboratory.

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O43

Use of Low Copy Number (LCN) DNA in Forensic Inference

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Since January 1999 the Forensic Science Service (FSS) has routinely carried out low copy number (LCN) DNA profiling in casework. To support this initiative, research has been carried out to discover the characteristics and limitations of LCN DNA by studying a series of well-defined evidence types, such as latent finger marks, and by measuring the propensity of donors to deposit DNA onto objects that they have touched.

The effect of various finger mark enhancement techniques on the recovery of DNA from latent marks has been investigated. Enhancement chemicals including cyanoacrylate, aluminium powder, iodine, ninhydrin, metal deposition, physical developer and gentian violet have been tested. Additionally the effect of exposure to several light sources has been examined. In general it was found that using a phenol chloroform extraction protocol, profiles could be obtained from marks after treatment with all of the chemical enhancements tested. However, recovery values varied between 33% and 100% of donor DNA profile depending on the chemical employed. Additionally it was determined that the best results were obtained when the time period between mark enhancement and DNA processing was less than 7 days. Latent marks were exposed to light sources for two time periods, generally there was little effect on DNA recovery even after an exposure time of 5 minutes. After exposure of 30 minutes two light sources were found to effect DNA integrity; Superlite and Polilight reduced the recovery of DNA to 65% and 33% of donor profile respectively. In these experiments the latent finger marks on porous or non-porous surfaces were deposited by one individual.

Experimentation using 29 subjects has shown that there is considerable variation in the amount of DNA that can be transferred onto an object by touching it. We have demonstrated that some individuals (defined as good shedders) can deposit a full DNA profile after contact of only 10 seconds with an inert object whereas others (defined as poor shedders) left behind very little DNA. Furthermore these results were reproducible, which indicated that some individuals can naturally and consistently deposit more DNA than others.

It is also possible for one individual to transfer DNA that has originated from another person (secondary transfer). This has been demonstrated by conducting 'hand-shaking' experiments, where the DNA of a good shedder could be transferred to an object by a poor shedder. These experiments were carried out under controlled circumstances designed to provide the best chance of obtaining a result. Preliminary experiments have also shown that tertiary transfer is possible. This could occur, for example, where an individual known to be a poor shedder transfers DNA previously deposited on one object to a different object.

To improve our understanding of low copy number DNA we have examined some well defined evidence types; for example donors were asked to wear and exchange identical wrist watches for a period of time in order to discover how long it took for the watch wearers DNA profile to replace that of the watch owner.

There are special considerations to be made when LCN cases are reported to the court. In particular, it is not possible to ascertain the time when a DNA profile was transferred onto an object (it may not be associated with the crime event itself – the possibility of innocent transfer must always be considered). The findings of transfer and persistence studies described in this paper help to define the limitations of the LCN technique.

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Are you collecting all the available DNA from touched objects?

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Since the principle of obtaining genetic profiles from touched objects became known[1] it has been well utilised world wide in police investigations. There is, however, often very little DNA on touched objects and investigators are not always able to generate a genetic profile from them. Here we investigate issues relating to the collection of material from touched objects in order to provide information that may assist improvement in obtaining genetic profiles from them.

Earlier studies[2,3] have discussed the benefits of appropriate targeting of samples and shown that multiple swabbings of the same area can significantly improve the recovery of available DNA. Tests comparing a number of different cleaning agents concluded that water was still the preferred moisturising solution[3]. Additional experiments have confirmed that there are significant differences in the amount of DNA deposited by different individuals but that there is no significant difference in the amounts deposited by the left and right hand of the one individual. There is also no significant difference in the amounts of DNA deposited by the same hand in six consecutive touches.

Further studies have shown that a significant proportion (20 to 76%) of the DNA that is collected by a cotton cloth / swab stick is lost during the extraction phase. The proportion lost was least with the smaller amounts from cotton cloth. Some of the loss is due to the extraction process (chelex, organic) and some is due to the type of matrix the sample is present on (cotton cloth versus swab stick), the amount of sample present in the matrix and/or the condition of the sample on the collection device (fresh versus dried). There were some significant differences in retrievability depending on the presentation of sample (dry, wet, cloth, stick). This was most pronounced between wet cotton cloth and dry swab stick with DNA being more readily retrieved from the former. Apart from wet cotton cloth, where chelex extraction outperformed organic extraction, there were no significant differences between the chelex and organic extraction methods used.

Significant proportions of DNA can also be lost whilst concentrating the DNA using Centricons® subsequent to extraction, especially when dealing with very small amounts of DNA. Much of what is lost was found in the top plastic section, membrane and rubber ring of the Centricon device.

Sample collection devices need to be sought from which the DNA is more readily retrieved after collection. As there is an interaction between the methods of collection and extraction, the various combinations of the new elements will need to be tested.

One must also be wary of quantitation results of trace amounts of DNA. Whilst methods such as Quantiblot™ are an improvement over some other methods, they are still only a guide. Their accuracy is limited. Tests conducted on regular experienced users found that there was variation both within and among users, and was most pronounced when quantitating lower amounts of DNA.

Through improved collection, extraction and quantitation techniques, we can make more of the available trace amounts of material from touched objects available for genetic profiling, thus increasing the chances of providing probative value to criminal investigations.

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O45

The Persistence of DNA under Fingernails Following Submersion in Water

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Two recent cases are described where STR profiling evidence, using the CTT system supplied by Promega, the SGM (Second Generation Multiplex) system, supplied by the Forensic Science Service and the SGM Plus system, supplied by Applied Biosystems, was obtained from DNA recovered from underneath the fingernails of two female victims. Each victim had been located submerged in water for different lengths of time.

In the first case, an intruder had killed the female victim by a number of blows to the head. She was later discovered about two hours after the incident submerged in water in the bath of her home. The person who found her released the water from the bath. In the second case the female suicide victim was located in Auckland City harbour, after having been observed jumping from a bridge approximately three hours earlier.

For each case, DNA may have transferred from a foreign source to the victim's fingernails. In the case of the home invasion victim, it was likely that the victim would have fought the intruder as young children were in the house at the time of the attack, and extensive bloodstaining in different areas of the house supported this opinion. In the second case, the victim had argued with her husband earlier in the day, allegedly scratching him.

This paper discusses the techniques used to recover the DNA from the fingernail samples and the DNA results obtained from each of the analyses. As the DNA results indicated the presence of foreign male DNA under the nails of each victim, the findings indicate the high priority that should be placed on the routine DNA analysis of deceaseds' fingernail samples, regardless of the scenes that they may be found in, including submersion in water.

O46

Real-time DNA quantification of forensic evidence materials

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A majority of the forensic evidence materials found at a scene of a crime contain minute amounts of DNA. Such evidence materials are saliva stains, shed hairs, skin cells and fingerprints on various items. We have developed a method to quantify the DNA amounts in various evidence materials. The quantification is based on a real-time 5' exonuclease determination assay using the ABI PRISM 7700 instrument (TaqMan). The assay will allow both nuclear and mitochondrial copy number determinations in a sample. The nuclear target in this quantification system is the retinoblastoma gene and the mitochondrial target is a tRNA gene in the coding region. This reaction is multiplexed in order to give an estimate of the total number of cells as well as the total number of mitochondrial DNA molecules in a particular evidence sample simultaneously. Based on the results can a proper selection of DNA analysis method such as nuclear or mitochondrial markers, multiplex or single PCR be made. Furthermore can the optimal amount of the extract to be taken without excessive waste of valuable DNA material be estimated.

The method has been optimized and tested on a number of different evidence materials in casework analysis. The assay has moreover been used to quantify the DNA amounts in a large variety of evidence materials of different size and quality as well as differently treated materials. These materials such as fingerprints, hair, blood stains, saliva stains and skin debris has been quantified according to their mitochondrial and nuclear DNA contents.

The efficiency of different DNA extraction procedures has also been evaluated using this TaqMan assay. Three different methods; Promegas Wizard kit for genomic DNA extraction, Chelex or hair digestion using protinase K and DTT have been used to purify DNA from forensic samples. DNA amounts in samples extracted with all the three different methods have been quantified and a significant difference was found. A difference in mitochondria per cell ratio was also seen. Furthermore have a number of different fingerprint detection procedures, that the police use to visualise fingerprints, been tested. DNA extracted from materials treated with ninhydrine, carbon powder and magnet powder has been quantified using the TaqMan assay. The result showed that fingerprints visualised with one of the methods contained notably less detectable DNA than the other techniques.

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O47

Chromosome X Haplotyping in Deficiency Paternity Testing – Principles and Case Report Presentation

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Chromosome X (ChrX)-linked microsatellite markers are powerful tools for parentage testing in special cases, mainly in deficiency paternity cases when the disputed child is female. ChrX haplotyping offers high individualisation potentials and can be used to prove the relationship between individuals in difficult cases. We carried out a kinship test in a case in which we could only rely on material from the cousins, as no biological material was available from the paternal and grandmaternal generation.

ChrX haplotypes can be easily established in male individuals as this chomosome occurs hemizygotic in the male sex. Thus, the haplotype of any ChrX linkage group can be deduced for female first-degree relatives, i.e. the mother and/or daughters of a male. Detailed knowledge on marker localisation is the basis for haplotyping application.

We used HPRTB and ARA protocols from the literature and established further ChrX STRs for kinship testing. The markers which were used in the case reported here were: DXS6807, DXS9895, DXS9902, DXS8378, DXS7132, ARA, DXS6800, DXS9898, DXS101, DXS7424, DXS7133, HPRTB, DXS7423 and DXS8377. A study involving more than 200 male blood samples failed to demonstrate a linkage disequilibrium between any markers under investigation.

The examination of the pedigree depicted here proved that the disputed offspring and two putative cousins shared an identical ChrX haplotype spanning the region Xp21 to Xq21. Hence, ChrX haplotyping confirmed the claim of the woman to belong to the deceased putative father's relationship.

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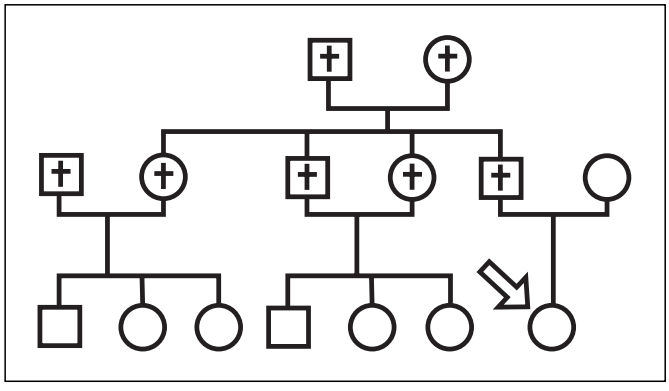

O48

Removal of PCR inhibitors with Thiopropyl Sepharose 6B from a bone from a body emerged from the sea

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Introduction

Attempts to amplify DNA from decomposed human remains by PCR are often hampered by the presence of inhibitors of *Taq*-polymerase. The current study investigated the use of Thiopropyl Sepharose 6B for the removal of PCR inhibitors from DNA extracted from a human thighbone from a decomposed body recovered after four years in seawater.

Materials and methods

An extensively decomposed bodypart was recovered in December 2000 by a trawler north of the Danish island Sejerø. Based on the clothing, it was anticipated that the remains were those of a 49 years old man, lost in the area during a fishing expedition in late 1996. To confirm this, DNA was extracted from the thighbone using proteinase K and phenol/chloroform extraction followed by concentration on Centricon 100 filters. For comparison, DNA was also extracted, in the same manner, from paraffin embedded tissue originating from the suspected victim. The amount of human DNA extracted was quantified by slotblot analysis followed by hybridisation with an alkaline phosphate conjugated D17Z1 probe. The DNA extract from the thighbone was treated with Thiopropyl Sepharose 6B (Sigma-Aldrich), previously shown to be effective to remove PCR inhibitors in clothing (Shutler et al., 1999). The DNA extracts were amplified with the AmpFlSTR SGM Plus kit (Applied Biosystems).

Results and discussion

The quantification of the DNA extracts revealed that the extraction procedure had resulted in sufficient DNA for a subsequent PCR analysis. Nevertheless, attempts to amplify DNA from the extract from the thighbone was fruitless, while amplification of the extracts from the paraffin embedded tissue yielded a full profile with the SGM Plus kit. After purification with Thiopropyl Sepharose 6B, amplification of the DNA extract from the thighbone was successful. The DNA-profiles obtained from the paraffin embedded tissue of the 49 years old man and the thighbone matched each other. We conclude that treatment with Thiopropyl Sepharose 6B was effective to remove PCR inhibitors from DNA extracted from a human bone in an extended state of decomposition.

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049

Grading of qualitative and quantitative responses in the PI proficiency survey of the College of American Pathologists for mailings in 1997–2000.

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The PI survey offered jointly by the American Association of Blood Banks and the College of American Pathologists provides biological samples for proficiency testing that mirror paternity casework samples. Grading of participant responses began in 1997 for qualitative results such as phenotypes consisting of discreet alleles (e.g. red cell antigens, enzymes and proteins, and PCR results). Beginning in 1998, quantitative responses (e.g. allele size estimates for RFLP systems) were included in grading. Grading of both qualitative and quantitative responses submitted by participants is peer based. For qualitative responses, good or acceptable responses are established by the consensus of a peer group (of a minimum of 10 participants) in which 90% agree. Grading of quantitative responses is also based upon consensus of a minimum peer group of 10 participants whose responses establish a mean and standard deviation. Each response is then measured against the mean and an acceptable range that is ±3SD from the mean. Any reponse lying outside this range is deemed an outlier. Following the first outlier pass, a new mean and acceptable range (i.e. ±3SD) are established from the remaining responses (assuming at least 10 participants remain in the peer group) and the remaining responses are again compared to the range to identify additional outliers. This grading scheme (e.g. the 3SDX2 scheme) has been used for grading quantitative responses such as band size estimates for RFLP systems (initiated in 1998) and for PI values (initiated in 2000) for all systems used for parentage analysis in the PI Survey. Paternity index responses for RFLP systems were grouped by locus regardless of the restriction enzyme used to increase the size of the peer group. In addition, analysis of ungraded, older data suggested that combining PI values by locus alone would not result in a high frequency of outliers because the spread of reported values was high already (Allen, et. al. Grading of quantitative data in the CAP/AABB parentage testing program. Prog. In Forensic Hemogenet. 8:602–605, 2000).

A review of graded participant responses over the three mailings of 1997–2000 are shown in Table 1 and reveal several things: First, it is clear from the data that identification of discreet alleles associated with PCR/STR systems has improved over the 4 year period shown and is presently more reliable and reproducible than is estimating the size of alleles associated with RFLP systems (Table 1). RFLP systems have remained fairly constant in terms of the frequency of phenotype outliers over the 4 years shown in the table. It is also clear from the data that the percentage of outliers for paternity index responses among RFLP laboratories is lower over the 4 years analyzed than responses from participants reporting paternity index values for PCR/STR systems. Moreover, outlier frequencies for paternity index values for DNA systems in general have not improved significantly over the past 4 years. Finally, an analysis of outlier frequency for the 4 year period indicates that whereas phenotype designations for PCR/STR systems is better than for RFLP systems, the opposite is true when one examines reported PI values.

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050

Results of the 2001 Paternity Testing Workshop of the English Speaking Working Group

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Since 1991, the English Speaking Working Group (ESWG) of the International Society for Forensic Genetics (ISFG) has, once a year, offered an exercise involving genetic analysis in a paternity case. Since 1998, laboratories performing immigration cases have been invited to treat the case also as they would do in an immigration case. Since the year 2000, the laboratories have been invited to calculate a paper challenge in addition to the paternity testing. Blood samples were sent to the laboratories together with information about the paternity case as well as the immigration case. Also, a questionnaire concerning the techniques and routines in the laboratories were distributed. The collated results have been published every 2nd or 3rd year (1–3).

Here, we present the results of the 2001 Paternity Testing Workshop. The evaluation includes concordance/discordance in typing results, collation of the systems used by the laboratories as well as methods used for purification of DNA, electrophoresis, measurement of sizes of DNA-fragments etc. Furthermore, we present a comparison of the requirements given by the laboratories to issue a report with an excluded man and with a non-excluded man, respectively. As laboratories used different systems for typing as well as different frequencies in calculations, comparison of calculated PI-values in the performed paternity testing was not possible. Therefore, the paper challenge constituted a valuable tool to compare calculations and to compare how laboratories dealed with inconsistencies, silent alleles as well as rare alleles. The results of the paper challenge are presented and discussed.

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O51

The GEDNAP blind trial concept

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The GEDNAP (GErman DNA Profiling group) blind trial concept, which was originally conceived as the name suggests for German laboratories only, was concipated in 1989/1990 to examine the interlaboratory variation of fragment length measurements and standardisation of the DNA single locus probes. This has now expanded and includes approximately 120 laboratories from all over Europe including Russia and only those STR systems are now included in the blind trial which reflect the present state-of-the-art in forensic DNA testing.

The success of the GEDNAP blind trial system lies in the concept of casework-oriented DNA stain testing coupled with a total participant-oriented feedback system for the presentation and evaluation of the results. This principle is also continued in the type and number of stains to be tested in subsequent trials, the selection of which is governed by the maxim of customer-demand. After successsful completion of the blind trial participating laboratories are issued a certificate which is recognised in all European countries as a validation of the competence of the laboratory for forensic DNA testing.

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O52

The proficiency testing program on DNA typing of the Spanish and Portuguese Working Group of the International Society for Forensic Genetics (GEP-ISFG)

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on behalf of the GEP-ISFG consortium

The Spanish and Portuguese Working Group of the International Society for Forensic Genetics (GEP-ISFG) (http://www.usc.es/gep-isfh/) comprises forensic genetics laboratories from Spain, Portugal, France and most of the Portuguese and Spanish speaking countries in America. A total of 225 forensic geneticists from 88 labs and 15 countries are members of the group.

Since 1992 the GEP-ISFG working group has been organizing annual collaborative exercises on DNA profiling with the aim of making progress in standardization and discussing technical and statistical problems in DNA profiling. Since 1995 a proficiency testing program coordinated by Quality Control Unit (National Institute of Toxicology-Ministry of Justice-Madrid) was carried out simultaneously with the GEP-ISFG collaborative exercises.

The number of participating labs increased continuously from 10 in the first exercise (GEP'93) to 77 in the last exercise (GEP'01).

The proficiency testing includes paternity cases and occasionally a criminal case. Statistical evaluation of the evidence was required in the last trials, including a theoretical paternity testing case with reported frequencies.

Despite de increasing number of labs, results remained quite satisfactory particularly in the last two trials. All the labs used PCR-based DNA polymorphisms (mainly autosomal STRs and Y-chromosome STRs) with an increasing number of markers until recently (now the tendency is for a reduction in the number of markers used). SLPs were used by a decreasing number of labs but the results indicated a good level of expertise despite the different protocols used. Good results were also obtained for mtDNA (reported by 35% of the labs in the GP'01 trial) despite the difficulties presented by the samples due the presence of length heteroplasmy in some trials.

Some statistical errors in statistical programs and calculations were detected in the theoretical paternity testing cases, especially when the difficulties of the case increase. This indicates that a greater effort must be made in this area and the corresponding actions to correct this problem were implemented.

In addition to the PT program the GEP-ISFG consortium has active working groups on nuclear DNA population databases (nuclear and mt), Y chromosome, quality management and accreditation, statistics, training&education and ethics.