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Mitochondrial DNA in Sensitive Forensic Analysis

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Abstract

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Genetic profiling is commonly performed on the autosomes using multiple DNA markers. Although routine forensic DNA analysis is robust and based on reliable technologies, samples with degraded or limited amounts of DNA often fail. In these cases, the analysis of mitochondrial DNA (mtDNA) can be very valuable due to the high copy number per cell. This thesis describes evaluation and modifications of existing technologies that are useful in forensic DNA typing, mainly focusing on mtDNA.

DNA quantities isolated from common evidence materials such as hairs, fingerprints and accessories were estimated using a real-time quantification assay. Knowledge of quantitative differences between materials can guide forensic scientists to perform the best analysis (Paper I).

The current mtDNA analysis is based on hypervariable region (HVI/HVII) sequencing, which is the most rigorous and time-consuming forensic DNA analysis. Therefore, we evaluated the possibility to exclude individuals by screening for non-matching samples using the rapid and easy mtDNA Linear Array Assay (Paper II).

The major disadvantage using mtDNA is the lower discrimination power compared to multiple nuclear DNA markers. In contrast to the nuclear genome, due to the uniparental (maternal) mode of inheritance, no individual has unique mtDNA. We investigated the possibility of increasing the discrimination power by using pyrosequencing technology to analyse parts of the coding region in addition to HVI/HVII (Paper III). Furthermore, the addition of coding mtDNA information was evaluated by comparing several recently published mtDNA coding region assays (Paper IV).

Mixtures of DNA are common in forensic genetics due to contribution of DNA from several individuals, contamination or heteroplasmy. To resolve mixtures we have developed a pyrosequencing-based assay for the accurate quantification of the mtDNA mixture components (Paper V).

In conclusion, this thesis describes several assays that are valuable in forensic genetics for DNA quantification, improved mtDNA analysis, and mtDNA mixture interpretation.

Keywords: Forensic genetics, Mitochondrial DNA, HVI/HVII, Nuclear DNA, Quantification, Real-time PCR, Immobilised SSO probe assay, Pyrosequencing, Discrimination power, Coding region, Mixtures

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*Till familj och vänner för att ni gör mig glad
och för att ni alltid finns där!*

*Optimisten har lika ofta fel som pessimisten,
men hon har mycket roligare.*

Okänd

List of papers

This thesis is based on the following scientific papers, which are referred to in the text by their roman numerals.

- I Nuclear and mitochondrial DNA quantification of various forensic materials**
Nilsson M.^{*}, Andréasson H.^{*}, Budowle B., Lundberg, H. and Allen M.
Forensic Science International 164:56-64 (2006)¹
- II Forensic Casework Analysis Using the HVI/HVII mtDNA Linear Array Assay**
Divne A-M., Nilsson M., Calloway C., Reynolds R., Erlich H. and Allen M.
Journal of Forensic Sciences 50:548-54 (2005)²
- III Forensic mitochondrial coding region analysis for increased discrimination using pyrosequencing technology**
Andréasson H., Nilsson M., Styrman H., Pettersson U. and Allen M.
Forensic Science International: Genetics In Press (2007)³
- IV Evaluation of mitochondrial DNA coding region assays for increased discrimination in forensic analysis**
Nilsson M., Andréasson H., Ingman M. and Allen M.
Manuscript
- V Quantification of mtDNA mixtures in forensic evidence material using pyrosequencing**
Nilsson M.^{*}, Andréasson H.^{*}, Budowle B., Frisk S. and Allen M.
International Journal of Legal Medicine 120:383-390 (2006)⁴

* These authors have contributed equally to the work.

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² Journal of Forensic Sciences, copyright © 2005 ASTM International.

³ Forensic Science International: Genetics, copyright © 2007 Elsevier.

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Related papers

- i Sensitive forensic analysis using the Pyrosequencing technology**
Nilsson M., Styrman H., Andréasson H., Divne A-M. and Allen M.
Progress in Forensic Genetics 11, International Congress Series 1288
(2006)¹ 625-627

- ii STR sequence variants revealed by Pyrosequencing technology**
Styrman H., Divne A-M., Nilsson M. and Allen M.
Progress in Forensic Genetics 11, International Congress Series 1288
(2006)¹ 669-671

The picture on the front page illustrates DAPI stained nuclear DNA found in the root of a plucked head hair.

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Abbreviations

ATP	Adenosine triphosphate
bp	Base pair
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
HUGO	Human Genome Organisation
HVI	Hypervariable region I
HVII	Hypervariable region II
LCN	Low copy number
mtDNA	Mitochondrial DNA
nDNA	Nuclear DNA
PCR	Polymerase chain reaction
rCRS	Revised Cambridge Reference Sequence
SNP	Single nucleotide polymorphism
SSO	Sequence-specific oligonucleotide
STR	Short tandem repeat

Introduction

DNA-based identity testing

Like a fingerprint, in most cases DNA is unique for a certain individual. Therefore, specific regions of the genetic makeup, known to be variable between individuals, can be analysed for DNA-based individual identification. This type of identification, also called genetic profiling, can be valuable on several different occasions.

Traces of DNA, commonly found at crime scenes, can be used to reveal the person who committed the crime as well as exonerate innocent suspects. In paternity testing and evolutionary studies, the relationship between individuals and populations is examined using DNA analysis. Identification of individuals by genetic profiling is also performed following mass disasters, such as the terrorist attacks of September 11, 2001 and the Asian tsunami in 2004 or when finding graves after conflicts ^{1,2}.

Furthermore, historical investigations can take advantage of DNA analysis. Ancient remains can be compared to relatives over generations. For example, skeletal remains from the assumed grave of the last Russian Tsar family, Romanov (1918), were investigated using DNA analysis ^{3, 4}. Following comparisons with relatives of the Tsar and the Tsarina, it was found with high likelihood that the remains are authentic and belong to the Romanov family. In addition, the DNA analysis of fossils from Neanderthal skeletons approximately 30,000 years old, found in caves on the southeastern boundary between Europe and Asia, support the hypothesis that Neanderthals were not on the direct lineage of modern man ^{5,6}.

History of DNA and forensic genetics

While people committing crimes and others trying to solve them have always existed, the history of forensic genetics is quite short. DNA typing was first introduced in forensic analysis in the middle of the 1980s but the DNA molecule was discovered long before that.

The discovery of DNA

Several names are worth mentioning in the early field of genetics and the discovery of DNA. Gregor Mendel is considered to be the founder of genetic science as he proved the basic principles of heredity by breeding experiments using peas in 1865 (presented in the paper "Versuche über Pflanzenhybriden", 1866 ⁷).

In 1869, Friedrich Miescher discovered a substance he isolated from the nuclei of white blood cells, which he called "nuclein" (initially described in the paper "Ueber die chemische Untersuchung von Eiterzellen", 1871 ⁸) ⁹. This substance later became known as nucleic acid.

Seventy-five years later, in 1944, Oswald Avery and his colleagues demonstrated for the first time that one type of nucleic acid, deoxyribonucleic acid (DNA), is the chemical element of heredity ¹⁰. Although it was not fully accepted at first, this discovery is now regarded as one of the most groundbreaking findings in the history of biology.

In 1953, James Watson and Francis Crick identified the double helix structure of the DNA molecule ¹¹. This Nobel Prize winning discovery (in 1962) was important knowledge leading to the understanding of basic mechanisms fundamental for life.

Some years later, in 1956, Joe-Hin Tjio and Albert Levan demonstrated that the majority of human DNA is densely packed in 46 chromosomes in the nucleus of the cell ¹² (*Figure 1*).

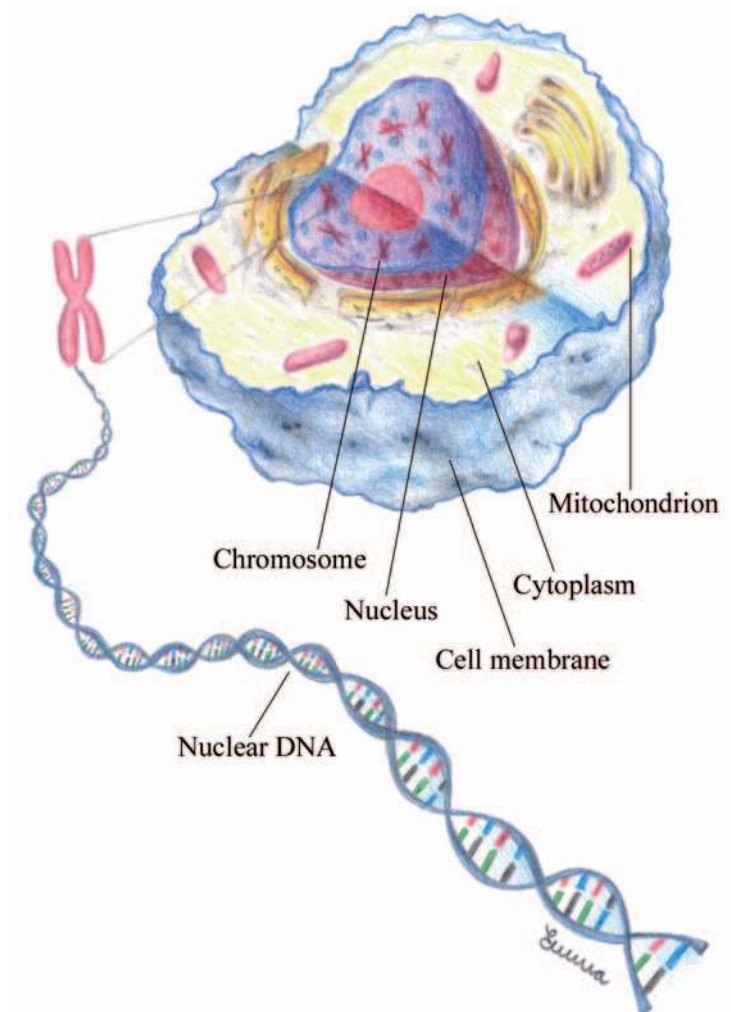


Figure 1. Illustration of a human cell with some of its organelles. Inside the nucleus, the nuclear DNA is condensed in chromosomes (46 in humans). The figure is made and provided by Emma Lindsten.

Inventions that are significant for forensic genetics

In 1985, Alec Jeffreys described DNA fingerprinting, the first DNA typing technology to be used for human identity testing¹³⁻¹⁵. DNA fingerprinting is based on the analysis of short (10 - 15 bp) core sequences shared by a subset of highly polymorphic minisatellites, or VNTRs (Variable Number of Tandem Repeats).

The first case resolved using DNA was an immigration case concerning a disputed identity in the United Kingdom in 1985. A year later, DNA profiling quickly spread worldwide and, in 1986, the first court conviction

based on DNA analysis was made in a double homicide case in Leicestershire, UK, referred to as The Blooding^{16,17}.

The main disadvantage in forensic genetics at this time was the large amounts of DNA needed. In 1985, Kary Mullis and co-authors presented a technique called Polymerase Chain Reaction (PCR) that is able to make several million copies of DNA from only a few DNA copies as starting material^{18,19}. This invention resulted in a Nobel Prize in 1993.

Following the invention of PCR, new PCR-based assays for DNA typing were introduced, replacing the methods for analysing minisatellite markers. Assays for another type of repetitive DNA marker, microsatellites, also referred to as Short Tandem Repeats (STRs), were developed. Fragment analysis of STR markers, which are standard today, was first described in the early 1990s and by 1996 the first multiplexed STR kits became available^{20,21}. In recent years, additional DNA analyses that can be useful under special circumstances have been developed, such as analysis of mitochondrial DNA and the Y-chromosome, as well as Low Copy Number (LCN) DNA testing. These assays contribute to make amplification and typing of mixtures, trace amounts or degraded DNA more straightforward.

The Human Genome Project

In the history of DNA research, the most recent breakthrough probably is the complete sequencing of the human genome. In 1990, the Human Genome Project (HGP) was initiated and the Human Genome Organisation (HUGO) was founded for the international coordination of genomic research. James Watson, one of the researchers that discovered the double helical DNA structure in 1953, was appointed to lead HGP.

In February 2001, two separate publications with the first draft of the human genome sequence were published^{22,23}. In April 2003, a completed version of the human genome reference sequence was announced (published in 2004), 50 years after the discovery of the double helix structure of DNA²⁴⁻²⁶. One of the biggest discoveries of the project was that the human genome consists of 20,000 to 25,000 protein coding genes, less than half the number of genes that were predicted²⁴. Only a few percent of the entire human genome of approximately 3 billion bp was found to encode proteins. Therefore, at least 95% of the genome is non-coding DNA, of which the function is currently poorly understood. A large fraction of the non-coding genome has proved to be under active selection and probably contains information about the regulation of gene expression²⁷.

The near complete DNA sequence, published in 2004, contained 2.85 billion nucleotides of the human genome and thereby a huge amount of information was made available. Subsequent to this, a lot of work remains to reveal what all this information can tell us about our species, *Homo sapiens*. Three key challenges for the future have been proposed, the identification of

(1) all genetic polymorphisms in the population, (2) all functional elements and (3) all interactions between genes and proteins^{24,25}.

The HGP project confirmed that repetitive sequences are often polymorphic and constitute a large part (>50%) of the human genome. Approximately 3% of the genome is found to consist of highly variable simple sequence repeats scattered throughout the human genome. Furthermore, the human genome contains millions of biallelic polymorphisms, Single Nucleotide Polymorphisms (SNPs). It is expected that about 10 million SNPs exist in the human genome of which many are linked variants forming a haplotype²⁸. The International HapMap project identifies and registers such common haplotype blocks in different populations^{29, 30}. Genetic variation among individuals, in the form of repetitive sequences or biallelic polymorphisms, can be used for human identification purposes.

DNA markers in forensic analysis

Analysis of fingerprints has been used in criminal investigations since the introduction of the fingerprint classification formula in 1901 (the Henry-Galton Classification System)³¹. The first genetic tool, although indirect, used for human identification was the ABO blood group determination, discovered in the beginning of the 20th century. This classical serological marker was determined by immunological methodologies. However, as the number of polymorphisms (or blood groups) is limited, blood grouping did not provide very informative results. In recent years, approaches based on highly polymorphic DNA markers have revolutionised the field of forensic science^{32,33}.

The human genome contains large regions of repeated DNA. Sequences of tandemly repeated DNA can be divided into three groups depending on their overall size; satellite DNA, minisatellite DNA and microsatellite DNA. The number and size of the repeat units varies between the groups. Satellites are composed of repeat units of a wide range of sizes and commonly span 100 kilobases up to megabases. Minisatellites, also called VNTRs, contain 10 to more than 1000 repeats with a size of 10 - 100 bp per repeat unit. Finally, microsatellites, also called STRs, usually contain less than 50 short repeat units, between 2 and 6 bp each³³.

In 1985, minisatellites or VNTRs were the first type of DNA marker to be introduced in forensic genetics, a method known as DNA fingerprinting. The tandem-repetitive regions were analysed using the RFLP technique (Restriction Fragment Length Polymorphism), in which the DNA molecule is cut at specific recognition sites. According to a method called Southern blot, the DNA fragments are then separated by size, denatured and transferred to a nylon membrane where they are detected by hybridisation to

radioactively labelled probes. DNA fingerprinting was first performed using multi-locus probes. Single-locus probes were later introduced, which were simpler to standardise and interpret. However, large amounts of DNA were required for both types of DNA fingerprinting and the technology was laborious^{32,34}.

The PCR revolution

The PCR technology was described in 1985 and is based on synthetic DNA replication^{18,19}. The reaction requires two primers flanking the region to be copied, free nucleotides and a DNA polymerase. The actual reaction involves three major steps; denaturation, annealing and extension. These steps are repeated in 30 - 50 cycles giving an exponential increase in DNA copies.

In the early 1990s, PCR-based DNA typing using microsatellites (STRs) was introduced in the forensic community^{35,36}. The STR markers are highly polymorphic and the number of repeats at each locus usually varies to a great extent. Fluorescent labelling and size separation using gel, or more recently, capillary electrophoresis is commonly used to detect length variation. The genotype is determined by the sizes of the two alleles that each individual carries, estimated by comparison to control allelic ladder markers^{21,37}. The nucleotide length of the fragment is then converted to the corresponding number of repeats for each allele. In 1997, 13 genetic markers were selected to be the core of the Combined DNA Index System (CODIS) developed by the FBI Laboratory, U.S. Two years later, in 1999, the U.K. and many European nations adopted 10 core loci as a standard, of which eight are overlapping loci with CODIS (FGA, TH01, VWA, D3S1358, D8S1179, D16S539, D18S51 and D21S11). Accordingly, five additional markers are used by CODIS (CSF1PO, TPOX, D5S818, D7S820 and D13S317). Moreover, two additional loci are used in the European standard (D2S1338 and D19S433). Today, several commercial kits have been developed for typing these loci for the purpose of individual identification. The number of alleles seen at the eight loci that overlaps between most of the kits varies from 15 to 89^{20,21}.

The commercial kits available today utilise multiplex analysis of multi-allelic STR markers located on different chromosomes for a high power of discrimination. Furthermore, the microsatellites are short compared to the VNTR markers, increasing the sensitivity. Since its introduction, multiplex STR analysis has become the golden standard and a valuable tool for forensic DNA typing used in most routine DNA analysis laboratories^{20,38}. There are several commercial kits for multiplex STR typing, e.g. PowerPlex[®] 16 (Promega Corporation) and AmpF/STR[®] Profiler Plus[®] ID (Applied Biosystems), typing 16 and 10 STR markers respectively. As mentioned above, most markers are shared between many of the kits,

allowing searches in international databases containing profiles of convicted felons^{20, 39}.

Single Nucleotide Polymorphisms (SNPs) are a type of DNA marker consisting of a change of a single nucleotide. Many different technologies are used for SNP analysis such as allele specific hybridisation, primer extension, oligonucleotide ligation and invasive cleavage^{40, 41}. Novel SNP genotyping methods, based on new platforms and chemistries are continuously being developed. The discrimination power obtained by analysis of a single SNP is lower compared to a single STR marker, so more markers are required. On the other hand, due to shorter amplicon sizes, analysis of SNPs has the advantage of a higher success rate if the DNA is degraded and in poor condition⁴².

Analysis of challenging samples

The DNA content obtained from a sample can vary enormously. One reason for limited DNA quantities can be that certain types of samples contain small initial amounts of DNA. In addition, degradation caused by time, environmental exposure to degrading agents and suboptimal storage conditions can reduce the DNA amounts available for analysis. Skeletal remains such as bones, teeth, hairs without roots and highly decomposed tissues are known to contain scarce amounts of intact DNA. Consequently, analysis involving this type of forensic sample, or even ancient DNA samples, is a major challenge.

For successful DNA typing by conventional autosomal STR analysis, 0.25 to 1ng of DNA is required^{38, 43}. Assuming that 3pg of DNA corresponds to 1 haploid cell, approximately 85 to 340 nuclear genome equivalents are needed⁴⁴. Efforts have been made to increase the possibility to analyse samples that contain limited amounts of DNA or are highly degraded. A critical factor influencing the success rate when amplifying degraded samples is amplicon size. The products in the conventional STR marker kits range in size from 100 to 450 bp, amplicon sizes that have proved to be unsuccessful in some cases^{38, 42, 45}. Thus, implementation of new markers or improved assays of existing markers can provide an increased possibility to amplify severely degraded DNA.

Since smaller amplicons are more likely to be amplified in samples containing degraded DNA, reduced-size markers (miniSTRs) have been developed. Some of the existing high molecular weight markers have been converted to miniSTR markers by reduction of the flanking regions outside the tandem repeat sequences^{42, 46-48}. However, the redesign of the primer binding sites is not possible for all conventional STR markers, due to a large range of allele sizes or unsuitable flanking regions for primer localisation. A clear advantage of using existing STRs with smaller amplicons (miniSTRs)

is their compatibility with established national DNA databases^{42, 49}. The lower limit for accurate analysis of the miniSTRs has been demonstrated to be approximately 31pg of DNA, corresponding to 10 genome equivalents, although 100 to 250pg (approximately 34 to 85 genome equivalents) is required for successful amplification of all markers⁴⁷. As very short PCR products (less than 150 bp) are preferable, new informative STR markers have been identified. The implementation of three new loci as European standard has been agreed upon by the European DNA Profiling group (EDNAP) and the European Network of Forensic Science Institutes (ENFSI)^{42, 50}.

An even more sensitive analysis can be achieved by SNP typing, as the amplicon sizes can be limited to around 50 bp. However, using SNPs requires more loci, approximately 50 SNP markers, to reach the discrimination power obtained with existing STR multiplexes (10 - 16 STRs)⁵¹⁻⁵³. In addition to these new or redesigned markers, other strategies can be used in special cases. To obtain a sensitive analysis, mitochondrial DNA (mtDNA) or Low Copy Number analysis (LCN) can be used. Furthermore, for a male-specific analysis of mixed samples, Y-chromosome analysis is often performed. These specialised typing assays for challenging samples are described in more detail in the following chapters.

Mitochondrial DNA

Forensic materials that are unsuccessfully typed for nuclear DNA (nDNA) markers are often more successfully analysed using the multicopy mitochondrial genome.

The mitochondrion is an organelle located in the cytoplasm of the cell and is thought to have originated from endosymbiosis of a proteobacteria into a primitive eukaryotic cell⁵⁴. The main function of these organelles is to produce cellular energy in the form of ATP in the oxidative phosphorylation process. During this process, electrons are transferred through protein complexes in the inner mitochondrial membrane, forcing protons (H⁺) out of the mitochondrial matrix thereby creating a proton gradient. The final step of oxidative phosphorylation is the generation of ATP by proton transport back into the matrix through Complex V, also called ATP synthase⁵⁵. In 1978, Peter Mitchell was awarded the Nobel Prize for the discovery of the electrochemical proton gradient, the chemiosmotic hypothesis, which is the mechanism of ATP synthesis^{56, 57}. Furthermore, Paul Boyer and John Walker shared half the Nobel Prize (in 1997) for the discovery of the ATP synthase mechanism⁵⁸⁻⁶⁰.

The sequence of the mitochondrial genome was first described in 1981⁶¹. The sequence was later corrected in 1999⁶² and is commonly referred to as the revised Cambridge reference sequence (rCRS). The mitochondrion contains its own double-stranded genome, consisting of 37 transcribed genes

located in the coding region. Of these genes, 13 code for proteins, 22 for tRNAs and two for rRNAs. The mitochondrial genome also consists of a non-coding control region, which contains the origin of replication for one of the mtDNA strands, the outer (heavy) strand (*Figure 2*).

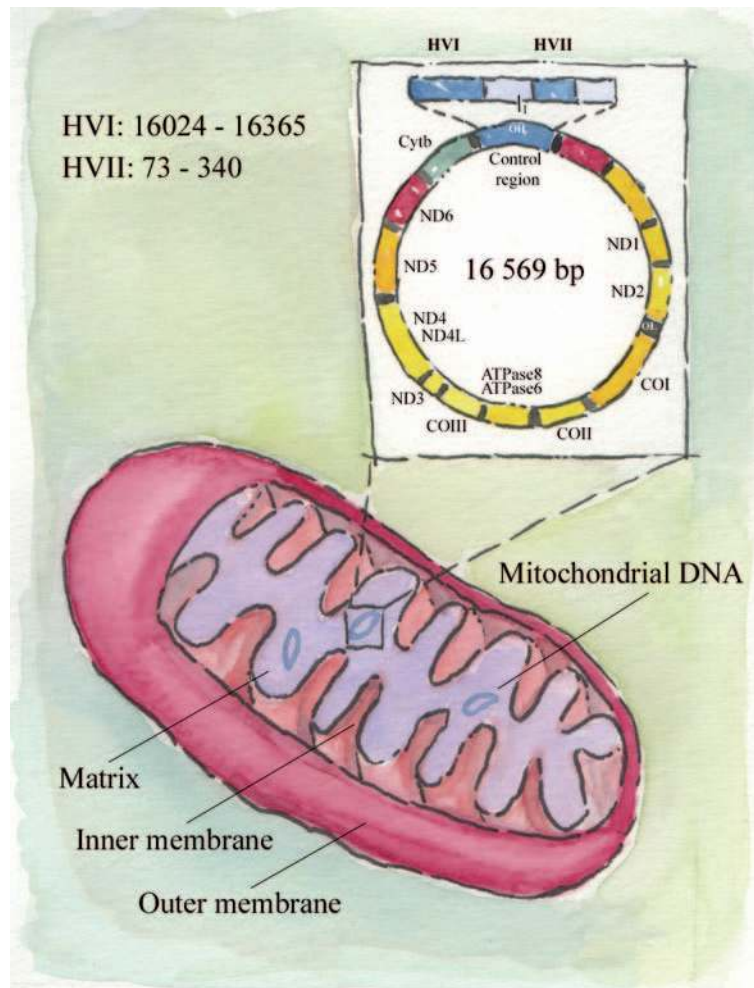


Figure 2. Overview of the mitochondrion. The mitochondrion is the only organelle in human cells that comprises DNA of its own (16,569 bp). The mitochondrial genome is found in the matrix inside the inner membrane. The figure is made and provided by Eva Uppsäll.

The number of mitochondrial DNA molecules per cell varies between different types of cells and tissues. It has been reported that each cell has on average 107 mitochondria and that each mitochondrion has between 1 to 15 mtDNA molecules with an average of 4.6. Consequently, each cell has approximately 500 copies of mtDNA, compared to two copies of nuclear DNA ⁶³. However, even taking the higher copy number into account,

mtDNA only comprises approximately 0.25% of total DNA in a cell. This is due to the significantly smaller size of the mitochondrial genome, consisting of 16,569 bp compared to the 3 billion bp of DNA in the nucleus²⁰.

The mitochondrial genome is circular, which may make it less prone to degradation by exonucleases^{20, 64}. Another characteristic feature of mtDNA is that it is entirely inherited from the mother⁶⁵; therefore all maternal relatives share the same mtDNA type. Also, since mtDNA has not been shown to recombine, it behaves as a single (clonal) locus and as a result no unique mtDNA profile exists.

Mitochondrial DNA has a very high mutation rate, approximately 10-fold higher compared to nuclear DNA, probably as a result of poor repair mechanisms as well as a decreased proofreading efficiency of the mtDNA polymerase^{20, 54, 66}. In addition, the mitochondrial genome is exposed to high levels of toxic free radicals produced during the oxidative phosphorylation, which is described in more detail below in the chapter “Mitochondrial DNA in ageing”. Another property of mtDNA compared to nDNA is the lack of protecting histone proteins, which further could contribute to the increased mutation rate observed in the mitochondrial genome^{54, 67}. When a mutation arises, a mixture of two types of mtDNA can be observed in an individual, a certain tissue, a single cell, or mitochondrion. This situation is known as heteroplasmy and is generally at such low level that it is normally not detected by standard methods⁶⁸. However, deleterious heteroplasmic missense mutations have been associated to disease. The proportion of normal and pathogenic types has been reported to affect the clinical outcome of the disease⁶⁹. Furthermore, the threshold for expression of disease by the same mtDNA mutation can differ among cell types⁵⁵.

Forensic mtDNA analysis

When conventional STR typing fails in forensic analysis, the advantage of the high mtDNA copy number per cell can be utilised. In samples that contain very small amounts of DNA or are severely degraded, mtDNA can often be used as an alternative source of genetic information. This method is highly sensitive with the capability to amplify and sequence small amounts of DNA. It has been shown that 30fg or approximately 10 mtDNA copies, less than the content of a single cell, can be sufficient for a successful analysis⁷⁰. Many legal cases involving different evidentiary materials, as well as population studies, have been conducted since the introduction of mtDNA analysis in the forensic field^{64, 70-74}. However, in comparison to the analysis of multiple nuclear markers, analysis of mtDNA results in a lower power of discrimination due to the lack of recombination.

Routine analysis of mtDNA is based on detection by Sanger sequencing of SNP variation in the two most variable regions in the genome, HVI and HVII. These hypervariable regions are situated in the non-coding control region (*Figure 2*). Generally, approximately 610 bp are sequenced in routine

forensic mtDNA analysis (HVI; 16024 to 16365 and HVII; 73 to 340) and the resulting nucleotide sequence is evaluated for deviations from the rCRS. It has been estimated that analysis of HVI/HVII in mtDNA results in 7 to 14 (8 on average) nucleotide deviations between unrelated Caucasian individuals ⁶⁴.

Different mtDNA lineages are commonly classified into haplogroups, defined by specific genetic variants. Haplogroups are often geographically related and the most common HVI/HVII type (the sub-haplogroup H1, identical to rCRS) is shared by 7% of the Caucasian population ^{72, 75-78}.

LCN analysis (low copy number)

Although an mtDNA profile can be very valuable in certain cases when conventional STR analysis fails, its low discrimination power remains as a main disadvantage. Therefore, extensive efforts have been made to achieve more sensitive nuclear DNA typing assays. One alternative strategy to analyse nDNA is called Low Copy Number (LCN) DNA testing. By increasing the number of PCR cycles in the routine assays from 28 to 34, a higher success rate for samples containing scarce amounts of DNA is obtained. LCN analysis can be performed using less than 100pg of input DNA, corresponding to 34 genome equivalents. The lower limit for LCN analysis has even been demonstrated to be 25 - 50pg of DNA, equivalent to approximately 8 to 16 genome equivalents ⁷⁹.

Y-chromosomal analysis

DNA markers located on the non-recombining region of the Y-chromosome (NRY) are passed from generation to generation without exchange of genetic material between chromosomes. Therefore, the Y-chromosome shows a uniparental inheritance pattern, similar to mtDNA although through paternal rather than maternal transmission. As with mtDNA analysis, this results in a lower power of discrimination compared to using unlinked autosomal markers. Nevertheless, analysis of polymorphic DNA markers on the Y-chromosome, STRs as well as SNPs, can be valuable in certain cases.

Samples taken after sexual assaults such as rape cases commonly consist of a mixture of female and male DNA. By analysing the male fraction using Y-chromosome markers, which in most sexual assault samples are present in minor amounts, the DNA profile from the male perpetrator can be determined. Furthermore, Y-chromosomal markers can be utilised to prove relationships in paternity testing, historical investigations as well in missing person investigations. In particular, this analysis is useful when only distant relatives on the paternal side are present as donors of reference material. Furthermore, due to the paternal inheritance, analysis of the Y-chromosome is helpful in evolutionary studies to perform phylogenetic analyses ⁸⁰. The

bi-allelic SNP markers on the Y-chromosome are regularly combined into haplogroups, just like mtDNA, while the multi-allelic STR markers are combined into haplotypes ²⁰.

The most common Y-STR markers used are the loci included in the “minimal haplotype”, proposed in 1997 (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385a/b), as well as two additional loci, later proposed by the Scientific Working Group on DNA Analysis Methods (SWGAM) (DYS438 and DYS439) ⁸¹⁻⁸⁵. As for the autosomal STR markers, commercial kits are available, e.g. PowerPlex[®] Y (Promega Corporation) and AmpF/STR[®] Yfiler (Applied Biosystems), typing 11 and 16 Y-STR markers respectively.

Technologies for detection of DNA markers in forensics

Fragment analysis of STR markers

The most common technology used today for analysis of STR markers is size separation by gel or capillary electrophoresis, using fluorescently labelled primers. The result of this analysis is the number of repeats on each allele and no information regarding the sequence is obtained. Two examples of partial DNA profiles following fragment analyses can be seen in *Figure 3*. The markers are usually amplified in one multiplex PCR reaction and all markers are separated by size simultaneously. Therefore, since fragments within the same size range will overlap, designing longer fragments for some of the markers can be required. A drawback with this strategy is the difficulty of amplifying the markers with longer fragments in the analysis of severely degraded DNA samples. The number of fluorescent dyes used for labelling the PCR products influences how many fragments that can be separated in each dye channel. The possibility to use more dyes, decreases the requirement of extending the sizes of the amplicons to avoid overlapping fragment sizes. Today a 5-dye detection system is most commonly used. This allows four of the dyes to be used for labelling the PCR products as one dye is used for the internal allelic ladder ^{20, 21}.

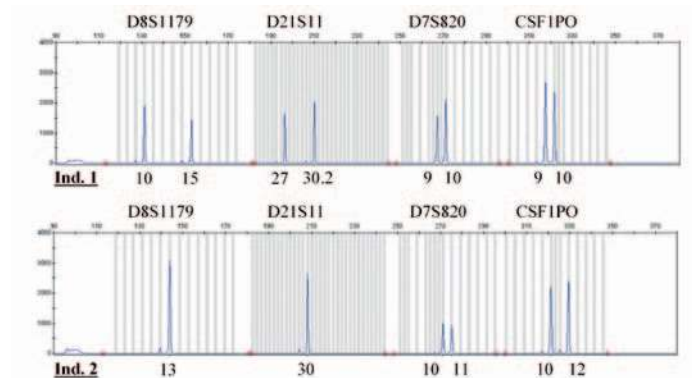


Figure 3. STR marker separation. Electropherograms illustrating size separation of four STR markers from two individuals. The y-axis shows the fluorescence intensity and the x-axis shows the molecular weight of the marker in number of bp. The AmpF/STR® Identifiler® kit (Applied Biosystems) has been used, analysing 16 markers. The electropherograms are provided by The National Board of Forensic Medicine, Department of Forensic Genetics, Linköping, Sweden.

Sanger dideoxy sequencing

In 1977, Sanger described a new method for determining the nucleotide bases in DNA⁸⁶. For this discovery, Frederick Sanger shared the Noble Prize in 1980. The technology is based on the incorporation of either deoxynucleotide triphosphates (dNTPs) or chain-terminating dideoxynucleotide triphosphates (ddNTPs), lacking a hydroxyl group at the 3' end, to the elongating DNA sequence. This results in the random generation of fragments of all various sizes, from only one base following the primer up to a thousand bases of sequence (Figure 4).

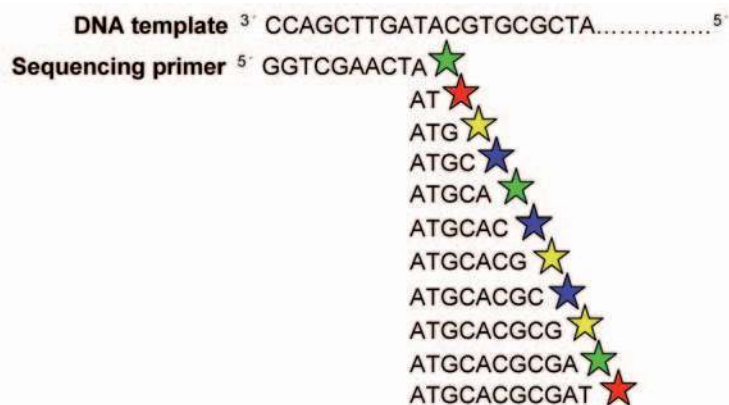


Figure 4. Principle of Sanger sequencing. Incorporation of ddNTPs terminates the elongation of the sequences at a given nucleotide, generating labelled fragments, each one base different in length (Dye Terminator™ chemistry).

Sanger sequencing can be performed by different labelling chemistries such as Dye Primer™ and Dye Terminator™ (Applied Biosystems). Using Dye Primer™ chemistry, four different sequencing reactions are performed, in which each contains a specific fluorescently labelled primer as well as one of the ddNTPs. Labelled sequencing primers hybridise to a universal tag sequence attached to the 5′ end of the PCR primers. Prior to size separation by gel or capillary electrophoresis, the four reactions are pooled together. The main advantage of this chemistry is that longer sequences with good quality can be achieved and the peaks are evenly distributed. For whole genome mtDNA sequencing (16 569 bp), an approach has been described that uses 24 overlapping PCR fragments together with the Dye Primer™ chemistry⁸⁷.

In the Dye Terminator™ chemistry, the four different ddNTPs are fluorescently labelled, one dye for each ddNTP (*Figure 4*). The sequencing is performed in one reaction and is therefore less time consuming and less labour intensive. Following electrophoresis, an electropherogram is generated as illustrated in *Figure 5*. The emission of the different fluorescent dyes is measured as the fragments pass a detector, creating peaks of different colours. The Human Genome Project (HGP) was completed using this sequence technology²²⁻²⁴.

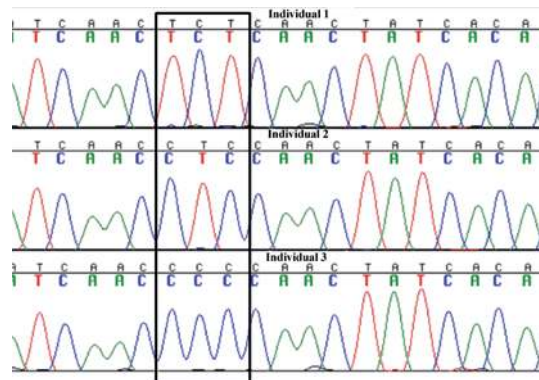


Figure 5. Sanger sequencing of mtDNA. Electropherograms generated by Sanger dideoxy sequencing of mtDNA extracted from three ancient bone remains found in a grave from the 10th century in Sigtuna, mid Sweden. The mtDNA sequences show 3 positions that differ between the individuals in the HVI region (16222, 16223 and 16224), indicated in the figure.

Pyrosequencing

The pyrosequencing technology is a sequencing-by-synthesis method that is based on four enzymatic reactions generating light^{88, 89}. Nucleotides are added one at a time, either in a cyclic or a sequence directed dispensation

order. If the nucleotide is complementary to the template DNA, the DNA polymerase enzyme will incorporate it and pyrophosphate (PPi) is released. ATP Sulfurylase converts the PPi to ATP, which is used by Luciferase to produce light. The last enzyme, Apyrase, degrades the excess of ATP and dNTPs before a new cycle begins. If the added nucleotide is not incorporated, Apyrase will degrade it without any production of light (*Figure 6*)^{90,91}.

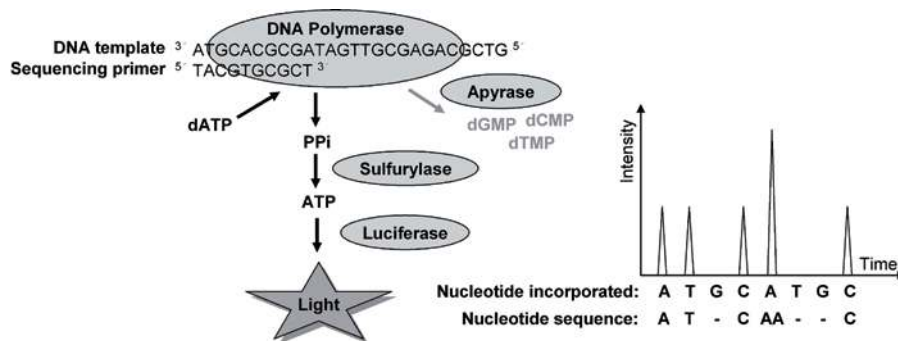


Figure 6. Principle of the pyrosequencing reaction. Illustration of the enzyme cascade that produces light proportional to the incorporated nucleotides.

The light that is produced in the pyrosequencing reaction is detected by a CCD camera and is illustrated as a peak in a pyrogram. As the light intensity is proportional to the number of incorporated nucleotides, a sequence that contains more than one identical nucleotide in a row (homopolymeric stretch) will create a proportionally higher peak (*Figure 7*).

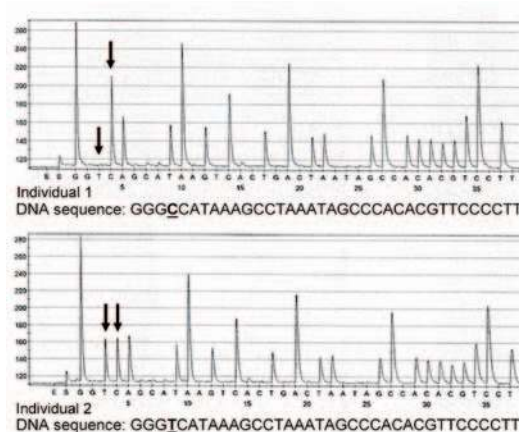


Figure 7. Pyrosequencing of mtDNA. Subsequent to nucleotide incorporation in pyrosequencing, light is produced and detected by a CCD camera generating peaks in a pyrogram. Pyrograms of mtDNA from two different individuals are shown in the figure, and arrows indicate a nucleotide difference obtained in position 16519.

The linear relationship between released light and the number of incorporated nucleotides allows accurate quantification, which is an advantage compared to Sanger sequencing⁹². An additional advantage of the pyrosequencing technology is that the DNA sequence immediately after the primer can be easily interpreted. By using Sanger dideoxy sequencing, the 20 first bases subsequent to the primer are often difficult to interpret due to the low molecular weight of short fragments as well as the interference of labelled nucleotides. Disadvantages of the pyrosequencing technology include limited possibilities of multiplexing, as well as a limited read length^{93, 94}. However, further improvements of the technology are constantly in progress. One example is the use of single-strand binding protein (SSB), which facilitates longer reads⁹⁵. Furthermore, the use of an alternative DNA polymerase, Sequenase, has proved to sequence homopolymeric stretches more successfully and has resulted in longer reads⁹⁶.

Several assays have been described for sensitive DNA analysis in criminalistics using the pyrosequencing technology. These assays determine a wide range of markers such as autosomal STRs, Y-chromosome markers (STR and SNP) as well as D-loop and coding mtDNA⁹⁷⁻⁹⁹.

Further developments and modifications of this technology have resulted in large-scale parallel pyrosequencing, referred to as 454 sequencing (454 Life Sciences). In this method, single-stranded DNA (ssDNA) is amplified by bead-based emulsion PCR (emPCR[™]). Sequencing is performed on millions of clonal copies of DNA fragments that are attached to beads¹⁰⁰. This approach has several advantages, for instance the large amount of sequences analysed in each run allows detection of rare DNA types. Furthermore, the sequence is derived from ssDNA, which makes it possible to observe the sequence from the actual template strand, as opposed to conventional sequencing. The 454 sequencing technology has been used for analysis of nuclear DNA and mtDNA of wholly mammoth remains¹⁰¹, ancient wolf remains¹⁰² as well as Neanderthal remains^{103, 104}.

Sequence-specific oligonucleotide probe hybridisation

One of the initial approaches to detect DNA polymorphisms based on PCR was oligonucleotide probe hybridisation. The technology was first based on sequence-specific oligonucleotide (SSO) probes that were hybridised to amplified DNA fragments attached to a membrane, called a dot blot¹⁰⁵. However, this was time consuming and further developments of the method resulted in the reverse dot blot assay, in which the SSO probes are immobilised to the membrane instead¹⁰⁶. Following the hybridisation, washing is performed under stringent conditions in order to allow detection of only the perfect matches between probe and target. As the DNA fragments are biotinylated during PCR, the enzyme conjugate streptavidin-horse radish peroxidase (SA-HRP) binds to the PCR products. By the

addition of a chromogenic substrate, a coloured precipitation becomes visible on the membrane (*Figure 8*).

Assays based on SSO probe hybridisation are commonly used as a pre-screening tool in forensic genetics, since they are less labour intensive, cheaper and less time consuming compared to many other DNA typing technologies¹⁰⁷⁻¹¹³. SSO typing has also been performed in many other areas such as disease association studies¹¹⁴, population genetics and evolutionary studies¹¹⁵ and for matching individuals before organ transplants¹¹⁶.

Hybridisation assays, other than SSO probe typing, have been developed, such as Dynamic Allele-Specific Hybridisation (DASH)¹¹⁷, LightCycler[®] and Molecular Beacons⁴¹. An additional method based on hybridisation is the TaqMan[®] assay, described in more detail below.

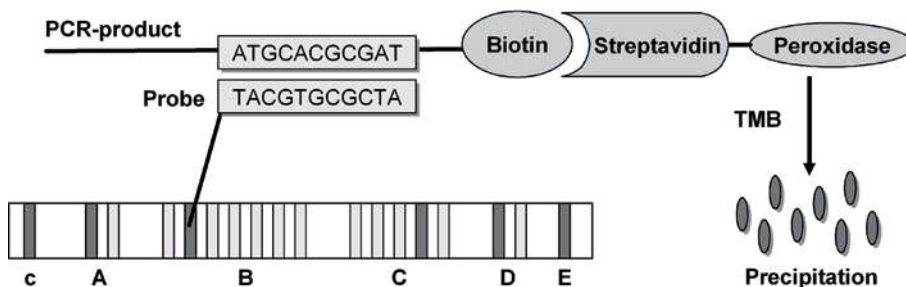


Figure 8. Overview of the linear array assay. Sequence-specific probes are attached to a membrane, to which biotinylated PCR products are hybridised. Following the addition of an enzyme conjugate and chromogenic substrate (tetramethylbenzidine, TMB), a coloured precipitation can be seen on the membrane where the probes with a perfect match are attached.

Quantification of DNA by real-time PCR, TaqMan[®]

Information regarding the amount of DNA (mtDNA and nDNA) recovered from a material is essential for further forensic DNA analysis. A quantification assay can guide the researcher to the optimal assay for further analysis for each specific sample. If there is enough DNA to perform nDNA analysis, which provides the highest discrimination power, a less discriminating and more labour intensive mtDNA analysis is avoidable. Furthermore, the optimal amount of DNA extract to be consumed in each analysis can be revealed by DNA quantification. As forensic materials are usually extremely limited, valuable DNA has to be used in such way that the minimum amount is used while ensuring that the analysis is informative and reliable and enough sample remains to permit replication.

The 5' to 3' exonuclease activity of the Taq DNA polymerase is utilised in the TaqMan[®] technology. A probe that contains a reporter fluorophore in the 5' end and a quencher fluorophore in the 3' end is hybridised to the DNA

template. For accurate quantification it is important that the target used is a single copy gene. As long as the two fluorophores are in close proximity, no fluorescence is detected. However, during strand elongation in the amplification process, the probe is cleaved and the reporter dye intensity increases with each cycle of the PCR^{118, 119} (Figure 9A).

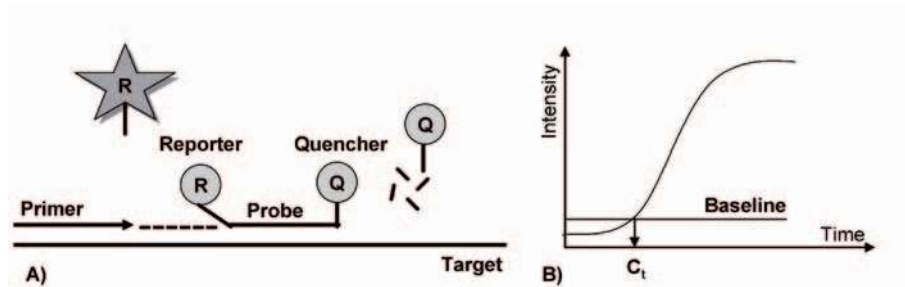


Figure 9. Principle of real-time quantification. A) Illustration of the Real-Time 5' exonuclease detection assay (TaqMan[®]) used for DNA quantification. The probe hybridised to the template is cleaved during elongation, causing separation of reporter and quencher, and thereby resulting in increased fluorescent emission intensity. B) Since the light is proportional to amplification, the cycle at which the emission rises above the baseline (C_t) can be used and compared to a standard curve to infer quantity.

The fluorescent emission intensity increases exponentially and is detected by a CCD camera. The specific cycle during the PCR at which the fluorescence reaches a threshold, is called the threshold cycle (C_t). If the sample contains low levels of DNA, a high number of amplification cycles will be required for detection, resulting in a high C_t . Therefore, the C_t is inversely proportional to the target concentration (Figure 9B).

To perform absolute quantification, serial dilutions of DNA with known concentrations are used to estimate the copy number in the sample. Quantification by real-time 5' exonuclease assays is in most cases extremely sensitive, allowing detection down to single copies⁹⁷. An example of mtDNA quantification of DNA recovered from a skull from the relic shrine in Vadstena Abbey can be seen in Figure 10. The study was performed according to Andréasson *et al.*⁹⁷.

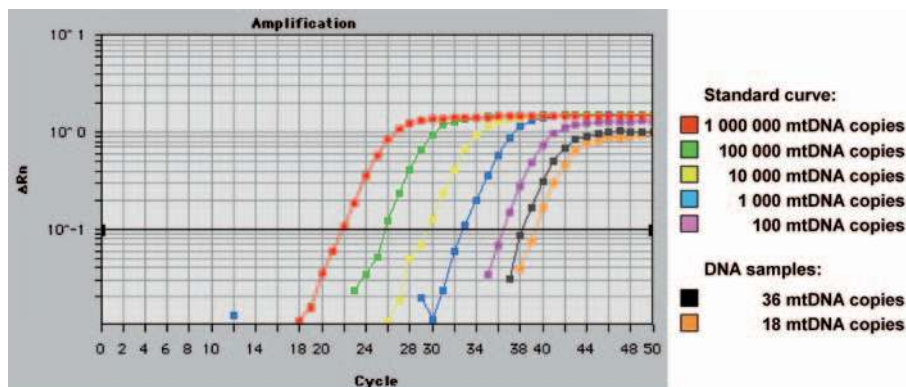


Figure 10. Quantification of mtDNA extracted from a skull preserved in the relic shrine in Vadstena Abbey. The amplification plot shows the result of two quantified DNA samples from the skull (18 and 36 mtDNA copies) as well as the standard curves of known DNA amounts. The y-axis shows the reporter emission intensity (in a logarithmic scale) and the x-axis shows the number of cycles.

There are methods other than quantitative real-time PCR (Q-PCR) used to determine DNA amounts. Some of those are hybridisation of radioactively labelled or biotinylated probes^{120, 121}, a system based on the Luciferase reaction (AluQuantTM)¹²², use of the intercalating dye PicoGreen[®]¹²³ as well as competitive PCR¹²⁴. However, these assays are not as sensitive as real-time 5' exonuclease assays and only detect nDNA copy number.

In addition, 5' exonuclease detection (TaqMan[®]) is commonly used for SNP typing, in which several probes are used for allele-specific discrimination¹²⁵.

Discrimination power and statistics

The discrimination power of the conventional analysis using multiplex autosomal STR markers is very high. Since in most cases, the different markers are located on different chromosomes, they can be regarded as unlinked, segregating independently of each other during meiosis. To estimate the random match probability the frequencies of the genotypes in a profile are multiplied. This is commonly described as the product rule²¹. This results in an average power of discrimination of 3.4×10^{17} for PowerPlex[®] 16 typing 16 STR markers, and 9.4×10^{10} for AmpF/STR[®] Profiler Plus[®] ID typing 10 markers²⁰. Therefore, analysis using nuclear DNA is preferred whenever possible, due to its high discrimination power.

The result obtained from mtDNA typing could be of three types: inclusion, exclusion or inconclusive. When the DNA profiles of evidence and reference are consistent at each nucleotide, the reference individual

cannot be excluded as the source of the sample and is therefore referred to as an inclusion. On the other hand, if there are at least two nucleotide differences between the samples, it can be excluded that the evidence is originating from the reference. The reason for the requirement of two nucleotide differences for exclusion is that although the samples originate from the same individual, it allows for the possibility of a sporadic mutation or heteroplasmy in one of the samples investigated. Therefore, if only one nucleotide difference is obtained between the two analysed samples, the result will be called inconclusive^{126, 127}.

When an identical mtDNA profile is shared between evidence and reference samples, the probability of a random match has to be evaluated, which is regularly done by the “counting method”. To demonstrate if the shared mtDNA profile is common or not, the number of times the particular sequence is observed in a database of unrelated individuals is counted⁶⁴. Since the size of the database could be a limiting factor, the frequency of an mtDNA profile is usually overestimated. The main reason for the rather low discrimination power is the uniparental inheritance pattern and lack of recombination. For this reason, mtDNA is treated as a single locus in the evaluation of typing results. As with the mitochondrial genome, no recombination occurs between the Y-chromosome markers, located in the NRY region. Therefore, the counting method is also used to estimate the frequency of a Y-chromosome profile.

Issues in forensic DNA typing

As mentioned above, there are several approaches to perform a highly sensitive analysis when DNA is scarce or degraded. This could be accomplished by miniSTR, mtDNA or LCN analysis. However, to ensure an optimal assay and reliable genetic profiling using these approaches, several issues have to be considered before routine implementation. These issues will be discussed in further detail in this chapter, emphasising those concerning mtDNA analysis.

DNA quantity and quality

Several environmental factors contribute to a rapid breakdown of DNA, including free radicals, water, high temperatures, nucleases and humidity. This degradation of DNA yields fragmented templates and is a major problem in forensic DNA typing. When too few intact DNA molecules are used in the analysis, partial STR profiles and allelic dropouts can be obtained^{20, 79}. Similar problems are associated with LCN analysis, mainly resulting from increased stochastic variation when such small amounts of DNA are investigated¹²⁸. Therefore, a duplication guideline, which involves duplicate

amplification and generation of a consensus profile, has been proposed to avoid misinterpretations when performing LCN analysis^{43, 79, 129}. Furthermore, by applying a statistical theory, LCN analysis takes stutters, allelic dropouts and contamination into account. Because of the ability to obtain DNA profiles from extremely small amounts of DNA, LCN typing has also been demonstrated to detect secondary transfer^{130, 131}. Thus, special caution should be taken in interpretation as well as in handling of the materials when analysis of low quantities of DNA is performed. Due to the uniparental inheritance of mtDNA, allelic dropout does not occur in mtDNA analysis. Nevertheless, standardisation of interpretation and nomenclature are highly important in mtDNA analysis and guidelines have been established^{126, 127, 132, 133}.

PCR inhibitors are quite common in evidence materials. Such inhibitory substances could be co-extracted with the DNA obtained from an evidence sample and can prevent PCR amplification in many ways. Common PCR inhibitors are melanin in hairs, textile dyes, urea and haemoglobin in red blood cells. To reduce the effect of inhibitors the DNA extract can be diluted. Addition of bovine serum albumin (BSA) as well as extra DNA polymerase has been shown to prevent or minimise the inhibition of amplification¹³⁴. Furthermore, separation of the inhibiting compounds from the DNA extract can be performed using certain spin columns containing filters for purification and concentration of the samples²⁰.

Contamination

Special care must be taken during forensic DNA typing to avoid contamination, especially when analysing small amounts of DNA. Although addition of more DNA polymerase and an increase in the number of PCR cycles usually improves analysis of low levels of DNA, it also makes the analysis even more prone to contamination^{50, 79}. Therefore, it is of great importance that the environment in laboratories performing mtDNA or LCN analysis is extremely clean. For mtDNA typing, which is a very sensitive technique for low levels of DNA, international recommendations regarding laboratory practice and handling of samples have been established^{126, 127, 132}. To screen for contamination, reagent blanks and negative controls should be analysed along with all samples. The quality of the DNA in most evidence samples is poor in comparison to DNA from laboratory personnel and reference samples. Therefore, evidence samples should be analysed before the reference samples to avoid DNA contamination of the evidence. Furthermore, all laboratory personnel should be DNA typed for comparison if contamination should occur²⁰. Analysis of ancient DNA, which is often regarded as the ultimate challenge in terms of contamination as well as degradation, has special restrictions and guidelines, which are discussed in a chapter below.

Mixtures

A DNA mixture is a sample consisting of DNA from more than one individual. Mixture obtained in an evidence material can have occurred prior or subsequent to sample collection. When forensic casework samples contain DNA from multiple contributors, the interpretation of DNA profiles is often complicated. In conventional STR typing, determination of the major and minor component in a mixture can be performed, but this requires careful interpretation and statistical evaluation^{135, 136}. However, in mtDNA typing, no reliable resolution of a mixture can be obtained by the routinely used Sanger sequence technology. The result of an mtDNA mixture is therefore usually called inconclusive. While reasonably accurate methods for mixture quantification have recently been developed, resolving mixtures can still be complicated. The number of contributors can be overestimated due to existence of heteroplasmy but also underestimated due to individuals sharing the same mtDNA haplogroup¹³⁷. To consider a sample a true mtDNA mixture, derived from multiple individuals, at least three positions in HVI/HVII (610 bp) should possess multiple nucleotides. Otherwise, the presence of more than one mtDNA type could be due to heteroplasmy²⁰.

Heteroplasmy

Heteroplasmy is a phenomenon where different mtDNA types are present within the same individual. Heteroplasmy might result from point mutations in the germline (during meiosis) or from somatic mutations (during replication in mitosis). Heteroplasmy is often regarded as the intermediate state between two homoplasmic DNA types. Subsequent to the occurrence of a mutation, more than one type of mtDNA can be present in a cell or tissue because of the multicopy feature of mtDNA. Through a process known as replicative segregation, the distribution of mtDNA types has been demonstrated to drift towards homoplasmy^{108, 138-140}. Heteroplasmy has been reported to differ between tissues, with the highest frequencies seen in muscle tissue, and has also been reported to increase with age¹⁰⁸. Heteroplasmy usually occurs in hotspot positions and more than one heteroplasmic site is rarely seen in the 610 bp normally sequenced in HVI/HVII^{20, 141}. Nevertheless, the presence of heteroplasmy can complicate the interpretation as well as improve the match probability in DNA identification. For this reason, interpretation guidelines provide recommendations of how to handle heteroplasmy^{126, 132}.

An example of a case where the finding of heteroplasmy actually improved the probability of a match is the Romanov case, explained in the chapter “DNA-based identity testing” above. The Russian Tsar Nicholas II and his brother (Grand Duke of Russia, Georgij Romanov) both displayed the same heteroplasmic position (16169C/T), supporting the identification⁴.

Two different categories of heteroplasmy can arise; single nucleotide (point) and length heteroplasmy. Length heteroplasmy, which is a likely result of replication slippage, is commonly seen in homopolymeric regions. Two C-stretch regions are located in the control region, one in HVI (16184 - 16193) and one in HVII (303 - 315). If no clear determination of the exact number of cytosines can be done, the heteroplasmic C-stretch region should be treated as having the same number of cytosines in all samples^{68, 72, 126}.

Pseudogenes

Pseudogenes can be described as a non-functional copy of a normal gene that has been slightly altered so it is no longer expressed¹⁴². Portions of the mitochondrial genome have been observed in the nuclear genome, described as nuclear mtDNA segments (Numts)^{142, 143}. These mitochondrial pseudogenes can be found evenly distributed across all human chromosomes and could complicate mtDNA analysis if they are amplified simultaneously with mtDNA. However, most of them are found to differ from modern mtDNA to some extent and since mtDNA is in excess of nDNA, the Numts rarely cause any problems^{20, 144, 145}.

DNA databases

Two types of DNA databases are available and used today. First, population databases with DNA profiles from a number of randomly selected individuals are available for frequency estimations. Second, DNA profiles collected from convicted offenders as well as DNA profiles recovered from evidence material in unsolved cases are stored in national DNA databases. Although both contain genetic information in the form of forensic DNA profiles, the use of these two types of databases is completely different.

Population DNA databases

When the DNA obtained from an evidence material matches the profile from a suspect, population databases are used to estimate the random match probability. For all markers used in conventional STR analysis, allele frequencies for different populations are stored in such databases⁸⁵. For a DNA profile created by a combination of genotypes detected on autosomal chromosomes, the size of the database is rarely a limiting factor due to recombination. Autosomal loci on separate chromosomes segregate independently during meiosis, which allows the genotype frequencies at each locus in a profile to be multiplied. However, as the population databases are comprised of allele frequencies, the sample sizes have to be sufficient to allow reliable estimations of genotype frequencies. For each

STR locus, 100-200 samples are commonly typed per population, which is usually sufficient²⁰.

In the case of an mtDNA inclusion, the frequency of that specific mtDNA type has to be estimated using mtDNA population databases. It has been demonstrated that certain mtDNA haplogroups are more frequent in some population groups, while nearly absent in others^{77, 78}. Therefore, the generation of mtDNA data from different populations is important for obtaining good estimations. Since the size of an mtDNA database can be a limiting factor, especially for rare mtDNA variants, it is essential to create large databases. There are several mtDNA HVI/HVII databases as well as databases of entire mtDNA genomes¹⁴⁶⁻¹⁵⁰. A first version of the comprehensive database, EDNAP mtDNA Population Database (EMPOP), was released in 2006 and will continuously grow and go through further development and evaluation¹⁵¹. As for mtDNA, the frequencies of Y-chromosomal haplotypes are estimated using the counting method because all markers are linked. A Y-chromosome Haplotype Reference Database (YHRD), which mainly contains the minimal haplotype, is available for the estimation of random match probability⁸⁵.

Quality of population DNA databases

The quality of the population databases used for the calculation of random match probabilities has been discussed thoroughly¹⁵²⁻¹⁵⁴. High quality databases are necessary to achieve accurate frequency estimations. Phylogenetic analyses to compare closely related mtDNA sequences from population databases have been performed and the result indicates that errors do occur^{155, 156}. Although rare, errors in mtDNA sequences can arise at many different stages in the analysis process. Errors can be due to technical or biochemical problems as well as misreading or contamination. If a population database contains erroneous sequences, it might reduce the estimate of the random match probability of the real DNA types¹⁵⁵. However, if inaccuracy takes place in a forensic case investigation, the impact on the estimate of how rare an mtDNA profile is, will in most cases be small¹⁵³. To avoid some of the errors that occur, quality controls should be performed on all generated data, with a final check for accuracy by phylogenetic analysis^{155, 157, 158}. Furthermore, highly automated assays will reduce the errors introduced by the human factor.

National DNA databases

In addition to population databases utilised for frequency estimation, there are also national DNA databases with DNA profiles of convicted offenders as well as DNA profiles developed from crime scene evidence. Most of the profiles stored in national DNA databases are based on STR markers

analysed using commercial multiplex STR kits. In many cases, the markers used are compatible between most countries. However, the legislation surrounding the national DNA databases differs between nations. In addition to conventional STR markers, mtDNA profiles have been introduced in similar databases for mass disaster reconstruction as well as missing person investigations²⁰. The first and largest national database of STR profiles is the UK National DNA Database, which was formed in 1995^{159, 160}. This database contains more than 3 million reference profiles¹⁶¹. These databases of convicted offenders and crime scene evidence generate matches and help solve several thousand cases each year. Moreover, international data exchange can assist in solving more crimes by international database searches through INTERPOL.

Although these databases have proved useful in many cases, there are ethical issues regarding the balance between the genetic integrity of the individual and the interest of the community³⁴. An increase in the number of DNA profiles stored in national DNA databases will most likely make the identification of recurrent offenders easier. It has been demonstrated that minor offenders might have a tendency to advance into committing more serious crimes. Therefore, identification of minor offenders has the possibility to prevent this progression^{159, 160}. An additional advantage of comprehensive national DNA databases is the possibility to perform immediate DNA identification of victims and missing persons following disasters^{20, 33}. Whether DNA profiles from all individuals in the world, including those convicted for any recordable offence or those only convicted for severe violent crimes, should be stored in databases, is discussed and, as mentioned above, legislation differs between countries.

Despite the benefit of national DNA databases in solving crimes, a fear of misuse of such data exists. The complete human genome harbours much information, including potential markers for diseases, which could be utilised by employers and insurance companies. The markers used for DNA profiling today are located in intronic sequence and do not identify variation in protein coding genes. Therefore, to our present knowledge, a DNA profile will not reveal any information about disease susceptibilities or behaviour. However, as the DNA samples are stored in many countries subsequent to DNA analysis, future technologies potentially can reveal such information if investigating new regions of DNA. Another concern regarding DNA databases is the fear of innocent individuals becoming suspects. On the other hand, wrongly accused individuals will be removed as suspects following DNA exclusion^{34, 159}. For these reasons, legislation concerning secure storage and proper use of the data and samples is highly important.

Analysis of mtDNA in other fields than forensics

In addition to the application of mtDNA analysis in forensics, several other disciplines study mtDNA variation. Some of these will be briefly discussed below.

Mitochondrial DNA in disease

Investigation of mtDNA is performed in medical science to study pathogenic mutations related to mitochondrial diseases, which affect the function of mitochondria^{69, 162}. Although the main pathogenic factor is the deficiency in oxidative phosphorylation resulting in insufficient ATP production, the effects of mitochondrial diseases vary to a great extent.

Several mtDNA mutations have been associated to diseases with a variety of symptoms such as movement disorders, muscle degeneration, blindness, deafness and dementia^{55, 163}. The degenerative symptoms most commonly affect tissues that require a lot of energy such as muscle and the nervous system. Examples of diseases associated to mutations in mtDNA include Leber's Hereditary Optic Neuropathy (LHON) leading to blindness, Neurogenic muscular weakness, Ataxia and Retinis Pigmentosa (NARP) resulting in progressive muscle weakness and Leigh's syndrome that is characterised as an often lethal progressive neurodegenerative disorder⁵⁵.

Heteroplasmy is commonly observed in diseases associated to mtDNA mutations. The proportion of the mutant mtDNA type in the tissue often determines the expression of a pathogenic mtDNA mutation, which is referred to as the threshold effect⁵⁵.

In addition to the mutations observed in the mitochondrial genome, some nuclear mutations have been identified as causing mitochondrial disease. Because most of the proteins active in oxidative phosphorylation are encoded by nDNA, additional mutations are likely to be found in the nuclear genome^{55, 164, 165}.

Mitochondrial DNA in ageing

During normal oxidative phosphorylation, reactive oxygen species (ROS) are formed as a by-product. These are usually removed (by the enzyme superoxide dismutase) in the mitochondria, thereby causing no damage. However, toxic hydroxyl radicals can be produced that damage the DNA in the mitochondria, which in turn could lead to decreased oxidative phosphorylation and increased levels of ROS¹⁶⁶⁻¹⁶⁸.

Mitochondrial diseases that have a delayed onset and progression could be explained by an accumulation of somatic mtDNA mutations with ageing, which cause a decrease of mitochondrial function. Accordingly, it has been demonstrated that mtDNA damage, point mutations as well as deletions, is

associated with age ^{167, 169, 170}. A recent mouse model, homozygous for a mutated mtDNA polymerase lacking the exonuclease activity that is essential for proofreading, revealed increased mutation levels as well as several age-associated features. The observed phenotypes were weight loss, curvature of the spine, osteoporosis, and hair loss among other characteristics, all commonly seen in elderly individuals. The mutated mice were also associated with reduced lifespan ¹⁷¹. Furthermore, dietary restriction in mice (40% less food) has illustrated that a lower metabolism preserves the activity of oxidative phosphorylation and lengthens lifespan ¹⁷²

Other factors than mtDNA damage have been proposed to increase the process of ageing, such as damage to the telomeres, which are long stretches of repeat structures (TTAGGG) flanking the ends of each chromosome in nuclear DNA. These structures are required for successful replication and, during cell division, the telomeric DNA gets shorter, which can accelerate ageing ¹⁷³. However, there are probably many factors causing ageing and more experiments will be needed to explain the complete mechanism.

Mitochondrial DNA in evolutionary studies

Analysis of mtDNA is commonly performed to investigate relationships between populations for determination of ancestry. The non-recombining property of mtDNA makes it a powerful tool for generating support of when and where the human species (*Homo sapiens*) originated. It has been shown by DNA analysis that the first modern humans evolved in Africa (mtDNA haplogroup L) 100,000 to 200,000 years ago. Furthermore, *Homo sapiens* have been estimated to spread from Africa, commonly described as the Out of Africa model, approximately 50,000 years ago ($\pm 20,000$ years) ⁷⁸.

In addition, analysis of mitochondrial as well as nuclear DNA obtained from the extinct species Neanderthal, dated to approximately 38,000 years before present, provides valuable information concerning evolution and the origin of modern humans ^{103, 104}. The time since Neanderthals and humans diverged is estimated to be about 500,000 years ago. It is also suggested that Neanderthals did not contribute any mtDNA to present humans ¹⁰³.

Mitochondrial DNA in the analysis of ancient remains

Chemical damage to DNA starts immediately after death and is largely dependant on the environment. Analysis of ancient DNA encounters several difficulties that complicate the analysis, such as severe degradation, minute amounts of DNA, the presence of inhibitors, contamination of other species and modern human DNA as well as post-mortem damage. The mitochondrial genome is commonly used in ancient DNA analysis due to the high copy number and sensitivity of the assay. To ensure the authenticity of ancient

DNA analysis, there are several requirements and special guidelines for performing such analyses ¹⁷⁴⁻¹⁷⁷.

Despite all obstacles, several successful DNA analyses of ancient materials have been performed, mainly using mtDNA. Examples of relatively old materials are a Neanderthal bone of approximately 38,000 years old, a woolly mammoth (33,000 years old), the 5000 year old mummified Tyrolean Ice Man, and a bone from the assumed evangelist Luke (A.D. 150) ^{101, 103, 104, 178-181}. More recent remains that have been analysed involve the putative son of King Louis XVI and Marie-Antoinette (1795), the legendary outlaw Jesse James (1882) and the Russian Tsar family, Romanov (1918) ^{3, 182, 183}.

Research in the field of ancient DNA constantly leads to the progress of the sensitive analysis of severely degraded DNA. What will be possible in the future, we can only imagine.

Present investigation

Aim

The aim of the publications in this thesis was to develop and evaluate technologies to be used in forensic genetics, mainly focusing on mitochondrial DNA. Some essential issues have been dealt with such as DNA quantification, pre-screening of samples by a hybridisation assay, mtDNA coding region analysis and mtDNA mixture resolution.

Paper I

Nuclear and mitochondrial DNA quantification of various forensic materials

Background

Information about the amount of DNA recovered from an evidence material could be highly valuable prior to DNA analysis. Quantification of nuclear and mitochondrial DNA copies can reveal which analysis method is the best alternative for a specific sample. The discrimination power will always be the highest for an analysis of unlinked autosomal STR markers or multiple SNP markers and, if the sample contains sufficient copies of nuclear DNA, this would be the most favourable approach. Moreover, a successful analysis should be performed so that a minimal amount of a valuable DNA sample is consumed. All this can be achieved using a real-time DNA quantification assay. In addition, real-time quantification can be utilised for increased knowledge concerning the variation in DNA content among commonly found evidence materials.

In this study, a previously developed real-time quantification assay¹⁸⁴ was used to evaluate the DNA content in different categories of commonly analysed evidence materials. Nuclear DNA copy number was determined in the root portions of plucked and shed head hairs as well as body hairs. Furthermore, epithelial cells from accessories and fingerprints, visualised using two different technologies, were evaluated for nDNA content. Also the number of mitochondrial DNA copies was estimated in epithelial cells

recovered from fingerprints, the root portions of plucked and shed head hairs, as well as in the more distal parts of hairs.

Results and discussion

The result of the study revealed large variations in DNA content between different categories of materials. The root part (first 10mm) of plucked head hairs contained on average 3,500,000 mtDNA copies and 25,800 nDNA copies. Quantification of the root part of shed head hairs revealed on average 45,700 mtDNA copies, while no nDNA copies were detected. Towards the more distal parts of the hairs, a consistent decrease in DNA amounts was observed. As expected, among head hairs the root part of plucked hairs contained most DNA copies, probably as a consequence of the different growth phases of the hairs¹⁸⁵. Three types of plucked body hairs were also evaluated for nDNA content. Eyebrow and beard hairs contained more nDNA copies on average compared to the first centimetre of plucked head hairs; 38,100 and 78,000 respectively. Arm hairs contain somewhat less DNA than the first centimetre of plucked head hairs, which on average contained 13,700 nDNA copies.

DNA was also quantified from epithelial cells collected from six categories of accessories and fingerprints visualised using magnetic powder and black powder. Among the accessories, the smallest amounts of DNA were observed in samples collected from rings and charms (approximately 100 nDNA molecules), whereas the highest amounts of DNA were detected in samples from earrings (on average 144,400 nDNA copies). For fingerprints, visualisation using black powder seems to result in higher DNA amounts (on average 170 nDNA and 12,000 mtDNA copies), compared to the magnetic black powder treated prints (on average 90 nDNA and 7300 mtDNA copies). Using this sensitive quantification assay, average DNA contents were revealed in many different common forensic evidence materials as valuable information for future DNA analysis.

Paper II

Forensic Casework Analysis Using the HVI/HVII mtDNA Linear Array Assay

Background

In cases where small amounts of DNA are found, the use of mtDNA is often more successful than analysis of nuclear markers, due to the high copy number per cell. Thus, analysis of mitochondrial DNA instead of nuclear DNA is required in certain investigations, with the consequence of lower

discrimination power. In a routine analysis of mtDNA, the two hypervariable regions, HVI and HVII, are analysed, usually by Sanger sequencing of both strands. This is a very time consuming, expensive and rigorous analysis. In cases when numerous samples are to be analysed, a faster and less laborious typing assay would be of great value, at least to be used as a pre-screening tool.

The HVI/HVII mtDNA Linear Array Assay (Roche Applied Science) has been investigated in this study. This assay is based on hybridisation of biotinylated PCR products to a panel of immobilised sequence-specific oligonucleotide (SSO) probes attached to a membrane. A retrospective study of 90 samples previously sequenced for mtDNA was performed. Based on these samples from 16 cases, the exclusion capacity of the linear array assay was estimated. Of the 90 samples, 57 were evidence materials (mostly shed hairs) and 33 were reference samples (mostly plucked hairs). Furthermore, a comparison of different versions of the linear array assay was performed between the older HVII linear arrays (17 SSO probes) and a more recent array covering HVI and HVII variants (31 SSO probes).

Results and discussion

Using the older HVII linear array, 41 of the samples were excluded, while 50 samples were excluded based on the new HVI/HVII probe panel. When sequence analysis of the D-loop was performed (by Sanger sequencing), 63 of the samples could be excluded. Thus, 27 of the samples were reported as inclusions following sequencing, involving 15 evidence materials and 12 suspects in nine of the 16 cases. Consequently, 50 of the 63 samples that were possible to exclude by sequencing (79%) could be excluded directly using the latest version of the linear array assay. Furthermore, the number of inconclusive results (a single nucleotide difference between samples) was reduced from 17 samples using the HVII assay to 7 using the HVI/HVII assay, while no samples yielded an inconclusive result in sequencing. In conclusion, although the exclusion capacity of the HVI/HVII linear array assay is somewhat lower than that obtained by sequence analysis, the assay provides a rapid tool for pre-screening of samples prior to sequencing.

Paper III

Forensic mitochondrial coding region analysis for increased discrimination using pyrosequencing technology

Background

The main disadvantage in analysing mtDNA is the low discrimination power obtained. The properties of the mitochondrial genome, such as lack of recombination in a uniparental inheritance pattern, result in that several people share the same mtDNA variants. In routine analysis of mtDNA, the hypervariable regions (HVI and HVII) are sequenced. Although, these two regions cover less than 4% of the complete mitochondrial genome, a large part of the variation among individuals is found within these two regions. Nevertheless, sequence analysis of HVI/HVII occasionally results in individuals sharing the same HVI/HVII type.

The coding region of the mitochondrial genome also harbours variable sites that can be utilised for individual identification. Several assays have been developed to study variation in the coding region of the mitochondrial genome, based on technologies such as SNaPshot analysis, microarrays and pyrosequencing^{75, 76, 97, 186-188}. In this study we investigated the increase in discrimination power by sequencing of additional variable regions in the mitochondrial coding region using a pyrosequencing-based assay.

Results and discussion

The assay includes 17 pyrosequencing reactions performed on 15 PCR fragments that cover informative coding regions of mtDNA (*Figure 11*). The average read length in the pyrosequencing analysis was 81 nucleotides, ranging from 20 to 122. In forensic analysis, guidelines advocate detection of at least two nucleotide differences to exclude that samples originates from the same source¹²⁷. Therefore a higher resolution for cases where only a single difference is found would be beneficial. In order to evaluate the resolution capability of the pyrosequencing coding system, 60 individuals displaying zero or a single difference to the rCRS in the HVI/HVII regions (0/1 individuals) were analysed. Another 75 individuals displaying at least two differences to the rCRS (2/2+ individuals) were included in the study.

In total, 52 SNPs were discovered in the 17 reactions, of which 18 were singletons. The 0/1 individuals also exhibit less variation in the coding region compared to the 2/2+ individuals, with 21 and 43 variable sites detected respectively, of which 12 were overlapping. For the 60 0/1 individuals, 48 (80%) could be resolved by additional SNPs when adding coding region information.

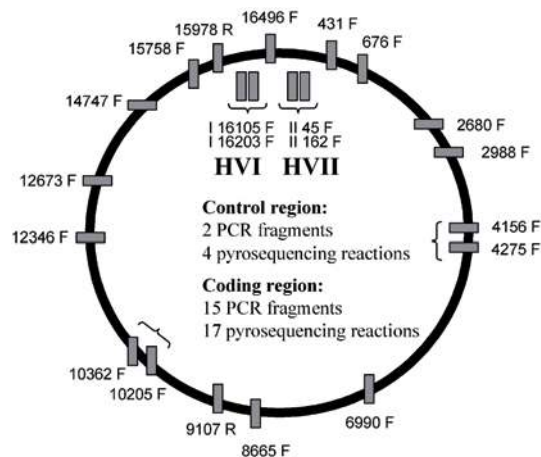


Figure 11. An overview of the mitochondrial genome indicating the fragments analysed by pyrosequencing in the hypervariable regions (HVI/HVII) as well as the coding part of the genome. The coding region assay consists of 15 PCR fragments analysed in 17 pyrosequencing reactions.

A case from 1952 is also described to illustrate the potential to resolve an evidence sample and suspect displaying a single nucleotide difference in HVI/HVII mtDNA analysis. Two additional variable positions were identified following mtDNA coding region analysis and a common source of the two samples was excluded.

This study demonstrates that additional variation was found in the coding region, which is useful as a complement to the routine analysis of the hypervariable regions HVI and HVII. Due to the fact that in most cases evidence materials are very limited, analysis of the reference samples can be performed prior to the evidence, to search for informative sites. This will save valuable evidence material DNA.

Paper IV

Evaluation of mitochondrial DNA coding region assays for increased discrimination in forensic analysis

Background

In routine mtDNA analysis, the HVI and HVII regions located in the control region are investigated. Due to the mode of inheritance and lack of recombination, multiple individuals share the same mtDNA type. To achieve higher resolution capacity among individuals, in recent years several assays detecting mtDNA coding variation have been described. The ability to

discriminate individuals with similar or identical HVI/HVII types using several of these mtDNA coding region typing assays was evaluated in this study.

495 Caucasian mtDNA sequences, obtained from a public database ¹⁴⁶, were divided into haplogroups and examined for the variation present in different regions (HVI/HVII, NonHV as well as the whole genome) subsequent to pairwise comparison. The four haplogroups mainly investigated were H (189 sequences), T (50), K (38) and U (31), which are all common among Caucasians. These 308 sequences, divided into the four haplogroups, were further analysed for the distribution of variation seen among individuals within the same haplogroup.

Furthermore, the 495 sequences were used to analyse the resolution capacity of eight previously published mtDNA coding region typing assays ^{75, 76, 187-192}. Pairwise comparisons were performed and the discrimination capacities for the different assays were evaluated. In all analyses, sequences from HVI (16024 - 16365) and HVII (73 - 340) were analysed together with the positions covered by the different coding region assays. This was done in order to mimic a routine analysis where the hypervariable regions are investigated first. The variation detected in the coding region is used as a complement to the HVI/HVII information. The different typing systems were also evaluated by similar analyses using 20 Swedish individuals sequenced for the complete mitochondrial genome.

Results and discussion

Large variations were observed in the comparison of pairwise differences within and between the different mtDNA regions as well as between the haplogroups. The NonHV region contained a larger number of nucleotide differences compared to the smaller HVI/HVII region. The largest difference observed between the haplogroups was seen in the routinely analysed HVI/HVII region. Haplogroup H and K were found to be the most similar in this region with the most frequent number of pairwise differences at only two, compared to T and U peaking at seven. These results, regarding nucleotide variation within different haplogroups, contribute to valuable understanding about the expected similarity between individuals originating from the same or different lineages.

The discrimination among the 495 mitochondrial genome sequences included in this study was evaluated based on eight different mtDNA coding region assays. The sequences were compared pairwise and the results revealed a large variation in discrimination power between the assays. Using the complete genome and the requirement of at least two nucleotide differences to be considered unique ^{126, 127}, 256 out of the 495 sequences could be excluded from all other sequences in this dataset. The assays were examined for the number of mtDNA types they were able to resolve out of

these 256 unique sequences. The HVI/HVII regions that are routinely analysed could only resolve 19% of the sequences, indicating that additional positions in the coding region could be very valuable in many cases. The system that proved to be the most discriminating, resolving 46% of the sequences, was the Andreasson 15seq assay, which utilises pyrosequencing to analyse 15 variable regions spread out in the coding region (Paper III) ¹⁸⁹. One reason for the high discrimination power of this test can be that the other assays are based on SNP typing and not sequencing, and thereby miss valuable low frequency SNP positions. The other assays could resolve between 20 and 30% of the unique sequences in this dataset.

Paper V

Quantification of mtDNA mixtures in forensic evidence material using pyrosequencing

Background

An mtDNA mixture can be observed due to contamination, heteroplasmy or DNA from more than one individual in a sample. In forensic DNA typing, determination of the minor and major contributor in mixtures is possible using the conventional analysis of multiplexed STRs. However, in mtDNA typing, which is commonly performed using Sanger sequencing, it is not possible to accurately quantify the different mtDNA variants in a mixture. Although mixtures can be detected by dideoxy sequencing, the nature of the sequencing chemistry prevents a reliable resolution of mtDNA mixtures. Therefore, in most cases the interpretation of a mixture will be inconclusive. Alternative technologies that have previously been described to resolve mtDNA mixtures have advantages and disadvantages. Some of these methods are denaturing high-performance liquid chromatography, fluorescently labelled restriction fragment analysis, cloning and quantitative real-time PCR ^{137, 193-195}.

In this study, an alternative method was developed to accurately quantify and resolve mtDNA mixtures. The method is based on pyrosequencing technology, in which the linear relationship between incorporated nucleotides and released light will allow accurate quantification. Seven highly variable positions, three in the control region and four in the coding region, were selected for mixture quantification. The PCR fragments used have previously been described in a pyrosequencing analysis assay, detecting variation in mtDNA control and coding regions ⁹⁷. Mixture standard series were prepared in 11 different ratios, for both the control region and the coding region. The four individuals used to produce the standards carried the following mtDNA variants: 73G, 146C, 195C and 73A,

146T, 195T for the control region mixture and 3010A, 7028C, 12372G, 14766C and 3010G, 7028T, 12372A, 14766T for the coding region mixture.

Results and discussion

The mixture standard series were quantified in four replicate experiments using the PSQ 96MA SNP Software version 2.02 (Biotage) and the proportion of each nucleotide added during synthesis was calculated. The measured mixture ratios were compared to the expected ratios, revealing high consistency. Although the quantification result for one of the seven positions (SNP 73) was not optimal, the final result could be improved after correction to the standard curve using the linear regression equation. For six out of seven SNPs, the previously designed PCR and pyrosequencing assays resulted in accurate quantification, whereas one of the SNPs (SNP 7028) required design of an internal pyrosequencing primer closer to the variable position. Furthermore, the mixture quantification assay was tested on three forensic evidence materials previously displaying mixtures in Sanger sequencing analysis of HVI/HVII mtDNA. The materials investigated were DNA collected from the lining of a wig, a bandage and a piece of duct tape. In each of the three evidence materials a mixture was observed in only one of the SNPs evaluated in this study, and the mixture ratios were determined to be 46% 73G, 19% 146C and 14% 195C for the three materials respectively. When forensic casework samples contain DNA from multiple contributors, it is usually noticed subsequent to the initial sequencing analysis. In the mixture quantification described in this study, the amplicons previously generated for sequencing are utilised to reduce the consumption of valuable DNA in addition to saving time. This mtDNA mixture quantification assay accurately interprets major and minor components in mixture samples and demonstrates an additional valuable application for the pyrosequencing technology.

Conclusions and future perspectives

Since the structure of the DNA molecule was determined in 1953, the sequence and structure of the human genome has been extensively investigated. During the past 20 years, the field of forensic genetics has developed dramatically and today DNA analysis is routinely performed in crime investigations. The DNA obtained from evidence material or from human remains after a disaster is frequently in poor condition. Therefore, the continuous development of novel methods as well as improvements of already existing technologies is essential to forensic genetics. At present, human identification is routinely performed using commercial kits based on autosomal STR multiplexes. Additional markers, currently in use under special circumstances, are autosomal SNPs as well as markers in the mitochondrial DNA and on the Y-chromosome.

This thesis describes the general use of DNA in forensic analysis, however the papers included focus on evaluation and modification of technologies for improved mitochondrial DNA analysis. For genetic profiling to be successful and informative, sufficient amounts of DNA have to be analysed. In Paper I, the DNA content, mtDNA as well as nDNA, obtained in commonly analysed evidence materials was estimated using a previously described real-time PCR method. A major disadvantage of mtDNA analysis is that sequencing is laborious and time consuming. Paper II illustrates the use of a rapid pre-screening assay (HVI/HVII mtDNA Linear Array Assay) that allows most of the samples to be excluded, and thereby reduces the sequencing efforts. Another disadvantage using mtDNA in forensic DNA analysis is the low discrimination power compared to conventional nDNA analysis. The addition of fragments in the mtDNA coding region, to be used as a complement to the information obtained in the routinely analysed HVI/HVII regions, was evaluated in Paper III. In recent years, this approach of analysing parts of the coding region has resulted in several publications describing novel mtDNA coding region assays. In Paper IV, eight of these assays have been evaluated for their increase in discrimination power. Finally, Paper V describes a mixture quantification assay using pyrosequencing technology to resolve mtDNA mixtures.

The future of forensic genetics will continue to take advantage of technical developments in molecular biology. Most research so far, has focused on improving the assays for individual identification. However, genetic information can also be utilised for other purposes. The possibility to

perform body fluid and tissue identification of DNA extracted from evidence material could be very valuable in forensic analysis. Multiplex mRNA profiling has been presented for detection of variable expression patterns in different tissues of forensic importance¹⁹⁶. Furthermore, determination of geographic origin can be used as a forensic intelligence tool by reducing the number of suspects in a forensic investigation. Genotypes obtained from several loci, called ancestry informative markers (AIMs), can yield population of origin to a certain probability¹⁹⁷. Finally, an exciting field for future research in forensic DNA typing is the genetics of appearance. It would be very valuable if prediction of phenotypic features could be used to create a genetically based phantom picture to be used as an investigative tool. For human phenotypes that have a strong genetic component, such as pigmentation, facial structure and eye colour, associations can be made between genotype and phenotype¹⁹⁸⁻²⁰⁰. However, most genes involved in the determination of appearance are so far unidentified and the interpretation will most likely be complicated due to involvement of multiple genes for most characteristics³⁴. Nevertheless, access to the genetic information obtained subsequent to the Human Genome Project as well as future technology development, suggests that this might be a possibility in the future.

Populärvetenskaplig sammanfattning

Tänk dig att ett grovt rån just har begåtts och rånarna har lämnat brottsplatsen i en flyktbil. Vem eller vilka är de skyldiga? En alltmer använd teknik som polisen tar till för att kunna spåra brottslingar är DNA-analys.

DNA, vår arvs massa

Man brukar säga att en människas utseende och egenskaper styrs av arv och miljö. Vår arvs massa, som även kallas för DNA, kan ses som en lång kod som består av fyra olika typer av byggstenar (A, T, G och C). En mängd information finns lagrad i denna kod, bland annat i form av gener som med hjälp av signaler i kroppen kan slås av eller på vid olika tillfällen. När en gen blir aktiv produceras en viss typ av protein enligt koden i vårt DNA, som sedan utför en viss funktion i kroppen. Två exempel på protein med olika funktioner är melanin som är ett pigment som ger färg åt hud och ögats iris, och proteinet keratin som används för att bygga upp hud, hår och naglar.

Det mesta av vårt DNA finns inuti cellens kärna, tätt packat i kromosomer. En människa har totalt 46 kromosomer, av vilka hälften har ärvts från vår mamma och hälften från vår pappa. Det DNA som finns i cellkärnan består av drygt 3 miljarder byggstenar som finns i dubbel uppsättning. Totalt kodar dessa 3 miljarder byggstenar i människans kärn-DNA för mellan 20 000 och 25 000 olika gener (*Figur 1*, s. 17).

Strukturen av DNA-molekylen beskrevs första gången 1953 av James Watson och Francis Crick, en upptäckt som gjorde att de fick Nobelpriset i medicin 1962. Mycket forskning har bedrivits kring uppbyggnaden och funktionen av DNA och flera Nobelpris har delats ut inom detta område. En av de största händelserna inom genetisk forskning var när man offentliggjorde koden för hela människans arvs massa. En näst intill komplett version av våra drygt 3 miljarder byggstenar presenterades 2003, precis 50 år efter det att DNA-strukturen beskrevs. Det enorma arbetet att få fram människans DNA-kod pågick under flera år och har kallats för HUGO-projektet (Human Genome Organisation). Resultatet av HUGO-projektet har medfört att all den information som finns lagrad i vårt DNA nu är tillgänglig. Trots att forskningen har gått fort framåt inom genetiken den senaste tiden, kan vi idag ännu inte förklara funktionen av all den information som finns lagrad i arvs massan. Men i takt med allt snabbare teknikutveckling och ökad

kunskap kommer många frågor om hur vi fungerar att kunna få ett svar i framtiden.

Det finns många skillnader i ordningsföljden av byggstenarna i DNA mellan olika individer. Man brukar säga att två obesläktade personer i genomsnitt skiljer sig åt i 100 000 byggstenar. Det finns flera olika typer av variationer i arvsmassan, där två vanligt förekommande skillnader är SNPs (Single Nucleotide Polymorphisms) samt STRs (Short Tandem Repeats). SNP-variationer är utbyte av enstaka byggstenar i DNA, som t.ex. när sekvensen AAGCTGCTAGT jämförs med AAAGCTGCTAGT. STRs är däremot längdvariationer i DNA, som bygger på skillnader i antalet upprepningar av en viss byggstenssekvens, som t.ex. DNA-sekvensen AGCTCATCATCAAAG som har 3 upprepningar av byggstenarna TCA, som kan jämföras med AGCTCATCATCATCAAAG som har 5 upprepningar av TCA.

Variationer i DNA-koden mellan individer kan tillsammans med andra faktorer förklara olikheterna mellan alla oss människor. Vissa positioner i vår arvs massa är extra känsliga för skillnader, vilket i värsta fall kan leda till sjukdom. Vår arvs massa innehåller till största delen DNA som inte kodar för några gener (cirka 95 %) och det är också i dessa delar av arvs massan som man hittar de flesta variationerna.

Rättsgenetik

Efter ett brott har begåtts granskar kriminaltekniker noga brottsplatsen och letar efter spår som rånarna lämnat efter sig. Det är näst intill omöjligt att inte lämna något DNA-spår efter sig alls, med tanke på att vi ständigt tappar hår och hudceller mm. Brottslingarna blir dock mer och mer försiktiga, eftersom de vet att DNA-analyser utförs, men trots det hittar man ofta DNA-spår på en brottsplats.

Det var först under mitten av 1980-talet som man började analysera skillnaderna i DNA mellan olika människor för att lösa brott. Tekniken man använde sig av då kallades för "DNA Fingerprinting" och byggde på att varje individ fick ett unikt mönster när man klippte sönder DNA:t i korta fragment, som kan liknas vid ett personligt DNA-fingeravtryck. Forskningen har gått framåt och dagens tekniker som används i rutin är betydligt känsligare, vilket gör att det endast behövs små mängder DNA för att få fram ett pålitligt resultat med ett högt bevisvärde. Dagens rutinanalys för att ta fram en DNA-profil bygger på att man analyserar mellan 10 och 16 olika STR-markörer, som ligger utspridda på olika kromosomer i kärn-DNA. För att en sådan typ av DNA-analys ska lyckas krävs det cirka 100 celler.

Lyckas man få fram en DNA-profil från ett bevismaterial, t.ex. en rånarluva som påträffats i en flyktbil, jämför man denna DNA-profil med DNA från olika misstänkta personer, för att ta reda på vem som kan vara den

skyldige till brottet. Får man en match, där DNA-profilen överensstämmer med en av de misstänkta, tar man fram ett värde på hur starkt detta bevis är. Bevisvärdet efter en rutinanalys är ofta väldigt högt och risken för att en match sker av slump brukar hamna mellan 1 av 1 000 000 000 och 1 av 100 000 000 000 personer.

DNA bryts ner med tiden, vilket kan gå olika fort beroende på vilken miljö som DNA:t befinner sig i. Chansen att hitta tillräckligt med DNA för en lyckad rutinanalys kan minska ganska snabbt t.ex. för ett bevismaterial som är slängt i en sjö eller som utsätts för höga temperaturer. I vissa fall räcker mängden kärn-DNA inte till för att få fram en fullständig DNA-profil. Förutom det DNA som finns i cellens kärna har vi också en annan typ av DNA som finns inuti cellens mitokondrier (mtDNA), som kan vara värdefull i dessa fall.

Mitokondrie-DNA

Mitokondrien är den enhet i cellen som producerar energi (ATP) så att alla funktioner i kroppen kan ske. Själva energiproduktionen, som också brukar kallas för oxidativ fosforylering, är beroende av gener som kodas av mitokondrie-DNA men även av kärn-DNA. Det DNA som finns inuti mitokondrien (mtDNA) är cirkulärt och mycket mindre än DNA:t som finns i cellkärnan, 16 596 byggstenar jämfört med drygt 3 miljarder i kärn-DNA (*Figur 2*, s. 23). En annan stor skillnad jämfört med det DNA som vi har i kärnan är antalet kopior; i en cell har vi mellan 500 och 2 000 kopior av mtDNA som är fördelat på flera olika mitokondrier, i jämförelse med kärn-DNA som finns i två kopior i kärnan i varje cell. Den fullständiga koden av mitokondriens arvs massa beskrevs första gången 1981.

Till skillnad från kärn-DNA som ärvs av både mamma och pappa har man kunnat visa att mtDNA bara ärvs från mamman via äggcellen vid befruktningen. Detta betyder att många individer delar exakt samma typ av mtDNA. Vi har alla samma mtDNA-variant som vår mamma, vår mormor, vår mormors mor o.s.v. förutom de spontana förändringar som ibland kan uppstå i en människas DNA under livet. Ytterligare en skillnad mot kärn-DNA är förekomsten av heteroplasm. Detta är när två olika varianter av byggstenar kan finnas i olika proportioner, t.ex. 30 % C och 70 % T i samma position i mtDNA. Även mtDNA kodar för gener, totalt 37 st. varav 13 kodar för proteiner. Förutom den kodande regionen består mtDNA även av en mindre icke-kodande region som består av drygt 1 000 byggstenar. Det är i denna region som man hittar de flesta skillnaderna i mtDNA mellan olika personer.

Det DNA som finns inuti mitokondrierna kan vara mycket värdefullt i brottsutredningar där man hittar väldigt små mängder av DNA. Detta beror främst på det stora antalet kopior av mtDNA (ca 1 000) som man hittar i

varje cell jämfört med kärn-DNA (2 kopior), vilket gör att man har cirka 500 gånger mer DNA att utgå från om man analyserar mtDNA. Vid en mtDNA-analys tittar man främst på två regioner som finns i mitokondriens icke-kodande del som kallas Hypervariabel region I och II (HVI och HVII). Ordningsföljden av de 610 byggstenarna som finns i HVI och HVII bestäms vanligtvis och de skillnader man tittar på är SNP-varianter. För att utesluta att ett bevismaterial kan komma från en misstänkt person krävs det att man hittar minst två SNP-skillnader i HVI och HVII. Detta beror på fenomenet att spontana förändringar kan uppstå i DNA under livet i olika vävnader vilket kan göra att två olika DNA-prov med en SNP-skillnad ändå kan komma från en och samma person. Bevisvärdet av en mtDNA-analys blir ofta lägre än vid en analys av kärn-DNA, eftersom flera individer kan dela identiskt mtDNA. Men i de fall där mängden kärn-DNA inte räcker till för en pålitlig analys kan mtDNA ändå vara av stort värde.

I Sverige utförs rutinanalys av kärn-DNA på Statens Kriminaltekniska Laboratorium (SKL) i Linköping, medan analys av mtDNA från bevismaterial görs i Uppsala i Marie Allens grupp, på Rudbecklaboratoriet.

Avhandlingen

Syftet med denna avhandling har varit att utvärdera och modifiera olika tekniker som kan vara värdefulla vid rättsgenetiska analyser. Främst har analyser av mitokondrie-DNA utvecklats. Titeln på avhandlingen kan översättas till ”Mitokondrie-DNA i känsliga rättsgenetiska analyser” och den är baserad på fem olika artiklar.

Artikel I

I den första artikeln har vi analyserat mängden kärn- och mtDNA i olika vanligt förekommande bevismaterial. Information om mängden DNA i ett material är mycket värdefull och kan ge vägledning om vilken typ av DNA-analys som är bäst lämpad. Tappade hårstrån är ett av de vanligaste bevismaterialen som man hittar på en brottsplats, varför vetskapen om mängden DNA i denna typ av material är extra intressant. Antalet kopior av mtDNA har analyserats utmed hela längden av såväl tappade som ryckta huvudhår och även mängden kärn-DNA har mätts i rot-delen. Mängden DNA analyserades också i några olika typer av kroppshår samt i material från fingeravtryck och smycken.

Resultatet visade stora variationer i DNA-mängd mellan de olika bevismaterialen. Den typ av hår som innehöll minst DNA var som förväntat tappade huvudhår. Detta beror troligtvis på att dessa hår går in i en fas där cellerna dör och DNA:t börjar brytas ner innan håret tappas. De ryckta huvudhåren innehöll i genomsnitt 77 gånger mer mtDNA (3 500 000 kopior)

jämfört med tappade hår (45 700 kopior) i den första cm av rot-delen. De ryckta hårrötterna innehöll dessutom 25 800 kopior av kärn-DNA, medan de tappade hårrötterna inte innehöll några kärn-DNA kopior alls. Vi kunde även se att mängden mtDNA minskade ju längre ut på hårstrå man mätte. Mängden kärn-DNA i de analyserade kroppshåren var i genomsnitt 13 700 kopior i armhår, 38 100 kopior i ögonbryn och 78 000 kopior i skäggrån.

Man kan ofta hitta DNA på smycken, eftersom man vanligtvis bär dem tätt inpå huden. Vi analyserade det kärn-DNA som kunde återfinnas på ringar, armbandsklockor, halsband/armband, glasögon, berlocker och örhängen. Bland de olika smyckena visade det sig att ringar och berlocker innehåller minst mängd DNA, cirka 100 kärn-DNA kopior, och örhängen mest DNA, i genomsnitt 144 400 kärn-DNA kopior.

Ett fingeravtryck är i sig individuellt för varje person och kan användas som bevis för att identifiera vem som har varit på t.ex. en brottsplats. För att ett fingeravtryck ska godkännas som bevismaterial krävs det dock att 12 unika punkter finns bevarade, vilket gör att fingeravtryck som är partiella eller utsmetade inte kan användas. Då finns alternativet att använda det DNA som lämnas i ett fingeravtryck som bevis istället. För att synliggöra ett fingeravtryck behandlas det vanligtvis med hjälp av olika kemikalier eller pulver. Vi analyserade mängden kärn- och mtDNA som kunde utvinnas från fingeravtryck behandlade med magnetpulver och järnpulver. Järnpulverbehandlade fingeravtryck innehöll ungefär dubbelt så mycket DNA (170 respektive 12 000 kopior av kärn- och mtDNA) som magnetpulverbehandlade fingeravtryck (90 respektive 7 300 kopior av kärn- och mtDNA).

Artikel II

Analys av mitokondrie-DNA är väldigt tidskrävande och dyrt. I den andra artikeln utvärderades en teknik där man med ett för-test eventuellt skulle kunna reducera andelen material som kräver en fullständig mtDNA-analys. Under den fullständiga analysen bestäms ordningsföljden av 610 byggstenar i HVI och HVII med hjälp av sekvensering, medan två olika varianter av för-testet som utvärderades i denna studie baseras på analys av enstaka SNP-positioner, 9 SNPs i HVII eller 18 SNPs i HVI och HVII. Totalt analyserades 90 olika DNA-prov (57 från bevismaterial och 33 från misstänkta) från 16 olika fall. Alla dessa material var tidigare analyserade och nu utvärderades om detta för-test kunde utesluta några material utan att den fullständiga, mycket tidskrävande analysen krävdes.

Av de 90 DNA-proven kunde totalt 63 st. uteslutas med hjälp av den fullständiga sekvensanalysen av mtDNA. De två olika varianterna av för-testet kunde utesluta 41 (9 SNPs) respektive 50 (18 SNPs) av de 90 proven. Detta betyder att 79 % (50 av 63 möjliga prov) kunde uteslutas med för-testet som analyserar 18 SNPs i HVI och HVII. Testets känslighet

utvärderades också och det visade sig att 100 kopior av mtDNA, vilket motsvarar mindre än en cell, gav ett pålitligt resultat som var enkelt att utläsa. Resultatet visar att ett för-test som detta kan vara mycket värdefullt, speciellt i de situationer då DNA från flera material kräver analys samtidigt, som t.ex. efter en masskatastrof.

Artikel III

Den största nackdelen med en analys av mtDNA är att risken för att få en match av slump mellan misstänkt och bevismaterial är högre jämfört med en rutinanalys av kärn-DNA. Detta beror på att det finns flera individer som delar samma mtDNA-variant. Vanligtvis analyseras 610 byggstenar i HVI och HVII, vilket motsvarar mindre än 4 % av hela mitokondriens DNA. Även i positioner utanför dessa två regioner, i den kodande regionen av mtDNA, finns det skillnader mellan individer. I den tredje artikeln har vi utvärderat möjligheten till ett ökat bevisvärde med hjälp av 15 variabla regioner i kodande mtDNA. Dessa 15 nya regioner i kodande mtDNA analyserades med hjälp av en relativt ny teknik som heter pyrosekvensering.

Den vanligaste mtDNA-varianten bland svenska personer i HVI och HVII förekommer hos cirka 7 % och kallas ofta för rCRS (revised Cambridge Reference Sequence). Till denna studie valdes 60 individer ut som inte gick att utesluta från sekvensen som är identisk med rCRS. Även ytterligare 75 individer som redan skiljde sig åt i minst två byggstenar från rCRS analyserades i kodande mtDNA. Totalt hittades 52 nya SNP-skillnader hos personerna i de 15 kodande mtDNA-regionerna. Bland de 60 individerna som inte gick att särskilja från varandra med analys av HVI och HVII kunde 80 % (48 individer) uteslutas från de övriga efter den extra informationen som kodande mtDNA gav.

I en äldre brottutredning, där man inte kunnat utesluta att ett bevismaterial kan komma från en misstänkt med hjälp av HVI och HVII, analyserades den kodande delen av mtDNA. Detta var ett fall från 1952 angående Biskop Dick Helanders eventuella delaktighet i propagandabreven som skickades ut till alla röstberättigade präster innan Biskopsvalet i Strängnäs. Analysen av HVI och HVII hade tidigare resulterat i en enda SNP-skillnad mellan DNA utvunnet från brevflikarna på propagandabreven och referensprov från Dick Helander, vilket inte är tillräckligt för en uteslutning. Med hjälp av kodande mtDNA-analys kunde dock två ytterligare SNP-skillnader identifieras, som styrker att det inte var Dick Helander som förseglade kuverten. Resultaten av denna studie visar att analys av kodande mtDNA kan vara värdefullt i fall där man inte kan särskilja DNA från olika material med analys av HVI och HVII.

Artikel IV

Även i den fjärde artikeln utvärderades den kodande delen av mtDNA som normalt inte analyseras i rutinverksamhet inom rättsgenetik. Hela mtDNA-sekvensen från 495 individer har använts i denna studie för att analysera antalet SNP-skillnader i olika regioner. Ett stort antal skillnader mellan olika personer hittades utanför HVI och HVII, vilket betyder att den kodande regionen kan vara mycket informativ i vissa fall.

Flera olika system som bygger på analys av variation i den kodande delen av mtDNA har beskrivits under de senaste åren och vi har utvärderat diskrimineringskapaciteten hos åtta av dessa system. Sekvensen från HVI och HVII användes tillsammans med de positioner som är specifika för varje system, eftersom kodande mtDNA oftast kommer att användas som ett komplement till de båda HV-regionerna. Antalet individer som var möjliga att urskilja från varandra med hjälp av hela mtDNA-sekvensen (alla 16 569 byggstenarna) var 256 av de 495 individerna. Av dessa lyckades sju av systemen urskilja mellan 20 och 30 % av individerna. Analys av enbart HVI och HVII kunde urskilja 19 % av de möjliga 256 personerna. Det system som beskrivs i den tredje artikeln i denna avhandling (pyrosekvensering av 15 kodande mtDNA-regioner) visade sig vara allra bäst på att särskilja individerna från varandra och hade en uteslutningskapacitet på 46 %.

Artikel V

Då man hittar en blandning av DNA från mer än en individ i ett bevismaterial kan resultatet ofta inte utvärderas när man analyserar mtDNA. En blandning kan bero på flera olika orsaker; materialet kan ha blivit utsatt för så kallad DNA-smitta (kontamination) redan på brottsplatsen eller på laboratoriet, materialet kan ha använts av mer än en brottsling vid själva brottet men även heteroplasm kan vara en anledning till att man upptäcker mer än en mtDNA-profil i ett material. Vi har i den femte artikeln använt pyrosekvensering för att analysera andelen av olika byggstenar i totalt sju olika SNP-positioner; tre positioner i icke-kodande mtDNA (73, 146 och 195) och fyra positioner i kodande mtDNA (3010, 7028, 12372 och 14766).

DNA från två individer med olika SNP-varianter i de sju positionerna blandades i proportioner från 0 till 100 % av respektive variant. Mätningen av SNP-varianterna gjordes med hjälp av pyrosekvensering och visade sig överensstämma väl med de förväntade proportionerna. Först krävdes dock optimering av två SNP-positioner för att ge tillförlitliga resultat.

Även fallmaterial som vid tidigare analys av HVI och HVII visat blandningar, analyserades med hjälp av detta nya DNA-test. De analyserade bevismaterialen var DNA utvunnet från en peruks, ett bandage samt en bit isoleringstape. Efter analysen av de blandade SNP-positionerna blev resultatet 46 % G i position 73 i DNA från peruken, 19 % C i position 146 i

DNA från bandaget samt 14 % C i position 195 i DNA från isoleringstapen. Denna studie visar på ytterligare ett värdefullt användningsområde av pyrosekvenserings-tekniken.

Framtidens forskning inom rättsgenetik

Mycket av framtidens forskning inom rättsgenetik kommer att handla om utvecklingen av metoder för att få fram resultatet av en DNA-analys snabbare än idag. En analys av mtDNA tar idag mellan tre och fem veckor, mycket beroende på alla rutiner och säkerhetsåtgärder som krävs för en pålitlig analys. I Sverige har man omhåkningsförhandlingar efter 14 dagar, vilket betyder att det skulle vara väldigt värdefullt att få fram en DNA-profil inom två veckor i framtiden. Det är även viktigt att utveckla nya tekniker inom rättsgenetik för att få DNA-analyserna känsligare än idag, vilket kan göra att ännu mindre DNA-mängder krävs för att få fram en användbar DNA-profil i en brottsutredning. Redan idag har studier visat att det är möjligt att analysera väldigt gammalt och nedbrutet DNA från t.ex. utdöda arter som mammut och neandertalare (över 30 000 år gamla material).

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References

1. Budimlija, Z. M., Prinz, M. K., Zelson-Mundorff, A., et al. *World Trade Center human identification project: experiences with individual body identification cases*. *Croat Med J* **2003**; 44(3):259-63.
2. Budowle, B., Bieber, F. R. and Eisenberg, A. J. *Forensic aspects of mass disasters: strategic considerations for DNA-based human identification*. *Leg Med (Tokyo)* **2005**; 7(4):230-43.
3. Gill, P., Ivanov, P. L., Kimpton, C., et al. *Identification of the remains of the Romanov family by DNA analysis*. *Nat Genet* **1994**; 6(2):130-5.
4. Ivanov, P. L., Wadhams, M. J., Roby, R. K., et al. *Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II*. *Nat Genet* **1996**; 12(4):417-20.
5. Krings, M., Stone, A., Schmitz, R. W., et al. *Neandertal DNA sequences and the origin of modern humans*. *Cell* **1997**; 90(1):19-30.
6. Ovchinnikov, I. V., Gotherstrom, A., Romanova, G. P., et al. *Molecular analysis of Neandertal DNA from the northern Caucasus*. *Nature* **2000**; 404(6777):490-3.
7. Mendel, G. *Versuche über Pflanzenhybriden*. *Verh. Nat.forsch. Ver. Brünn* **1866**; 4:3-47.
8. Miescher, F. *Ueber die chemische Untersuchung von Eiterzellen*. *Med. Chem. Unters* **1871**; 4:441-460.
9. James, J. *Miescher's discoveries of 1869. A centenary of nuclear chemistry*. *J Histochem Cytochem* **1970**; 18(3):217-9.
10. Avery, O. T., MacLeod, C. M. and McCarty, M. *Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Inductions of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III*. *J Exp Med* **1979**; 149(2):297-326.
11. Watson, J. D. and Crick, F. H. *The structure of DNA*. *Cold Spring Harb Symp Quant Biol* **1953**; 18:123-31.
12. Harper, P. S. *The discovery of the human chromosome number in Lund, 1955-1956*. *Hum Genet* **2006**; 119(1-2):226-32.
13. Gill, P., Jeffreys, A. J. and Werrett, D. J. *Forensic application of DNA 'fingerprints'*. *Nature* **1985**; 318(6046):577-9.
14. Jeffreys, A. J., Wilson, V. and Thein, S. L. *Hypervariable 'minisatellite' regions in human DNA*. *Nature* **1985**; 314(6006):67-73.
15. Jeffreys, A. J., Wilson, V. and Thein, S. L. *Individual-specific 'fingerprints' of human DNA*. *Nature* **1985**; 316(6023):76-9.
16. Wambaugh, J. *The Blooding: The True Story of the Narborough Village Murder*. Bantam Press **1989**.

17. Wong, Z., Wilson, V., Patel, I., et al. *Characterization of a panel of highly variable minisatellites cloned from human DNA*. *Ann Hum Genet* **1987**; 51 (Pt 4):269-88.
18. Mullis, K., Faloona, F., Scharf, S., et al. *Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction*. *Cold Spring Harb Symp Quant Biol* **1986**; 51 Pt 1:263-73.
19. Saiki, R. K., Scharf, S., Faloona, F., et al. *Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia*. *Science* **1985**; 230(4732):1350-4.
20. Butler, J. M. *Forensic DNA typing*. Elsevier **2005**.
21. Butler, J. M. *Genetics and genomics of core short tandem repeat loci used in human identity testing*. *J Forensic Sci* **2006**; 51(2):253-65.
22. Lander, E. S., Linton, L. M., Birren, B., et al. *Initial sequencing and analysis of the human genome*. *Nature* **2001**; 409(6822):860-921.
23. Venter, J. C., Adams, M. D., Myers, E. W., et al. *The sequence of the human genome*. *Science* **2001**; 291(5507):1304-51.
24. *Finishing the euchromatic sequence of the human genome*. *Nature* **2004**; 431(7011):931-45.
25. Collins, F. S., Green, E. D., Guttmacher, A. E., et al. *A vision for the future of genomics research*. *Nature* **2003**; 422(6934):835-47.
26. Collins, F. S., Morgan, M. and Patrinos, A. *The Human Genome Project: lessons from large-scale biology*. *Science* **2003**; 300(5617):286-90.
27. Waterston, R. H., Lindblad-Toh, K., Birney, E., et al. *Initial sequencing and comparative analysis of the mouse genome*. *Nature* **2002**; 420(6915):520-62.
28. Gabriel, S. B., Schaffner, S. F., Nguyen, H., et al. *The structure of haplotype blocks in the human genome*. *Science* **2002**; 296(5576):2225-9.
29. *A haplotype map of the human genome*. *Nature* **2005**; 437(7063):1299-320.
30. Thorisson, G. A., Smith, A. V., Krishnan, L., et al. *The International HapMap Project Web site*. *Genome Res* **2005**; 15(11):1592-3.
31. Knowles, A. M. *Aspects of physicochemical methods for the detection of latent fingerprints*. *J. Phys. E: Sci. Instrum.* **1978**; 11.
32. Carracedo, A. and Sanchez-Diz, P. *Forensic DNA-typing technologies: a review*. *Methods Mol Biol* **2005**; 297:1-12.
33. Varsha. *DNA fingerprinting in the criminal justice system: an overview*. *DNA Cell Biol* **2006**; 25(3):181-8.
34. Jobling, M. A. and Gill, P. *Encoded evidence: DNA in forensic analysis*. *Nat Rev Genet* **2004**; 5(10):739-51.
35. Hammond, H. A., Jin, L., Zhong, Y., et al. *Evaluation of 13 short tandem repeat loci for use in personal identification applications*. *Am J Hum Genet* **1994**; 55(1):175-89.
36. Urquhart, A., Kimpton, C. P., Downes, T. J., et al. *Variation in short tandem repeat sequences--a survey of twelve microsatellite loci for use as forensic identification markers*. *Int J Legal Med* **1994**; 107(1):13-20.
37. Gill, P., Koumi, P. and Allen, H. *Sizing short tandem repeat alleles in capillary array gel electrophoresis instruments*. *Electrophoresis* **2001**; 22(13):2670-8.

38. Cotton, E. A., Allsop, R. F., Guest, J. L., et al. *Validation of the AMPFISTR SGM plus system for use in forensic casework*. *Forensic Sci Int* **2000**; 112(2-3):151-61.
39. Tomsey, C. S., Kurtz, M., Kist, F., et al. *Comparison of PowerPlex 16, PowerPlex1.1/2.1, and ABI AmpFISTR Profiler Plus/COfiler for forensic use*. *Croat Med J* **2001**; 42(3):239-43.
40. Budowle, B. *SNP typing strategies*. *Forensic Sci Int* **2004**; 146 Suppl:S139-42.
41. Sobrino, B., Brion, M. and Carracedo, A. *SNPs in forensic genetics: a review on SNP typing methodologies*. *Forensic Sci Int* **2005**; 154(2-3):181-94.
42. Dixon, L. A., Dobbins, A. E., Pulker, H. K., et al. *Analysis of artificially degraded DNA using STRs and SNPs--results of a collaborative European (EDNAP) exercise*. *Forensic Sci Int* **2005**.
43. Gill, P. *Application of low copy number DNA profiling*. *Croat Med J* **2001**; 42(3):229-32.
44. Higuchi, R., von Beroldingen, C. H., Sensabaugh, G. F., et al. *DNA typing from single hairs* *Nature* **1988**; 332(6164):543-6.
45. Krenke, B. E., Tereba, A., Anderson, S. J., et al. *Validation of a 16-locus fluorescent multiplex system*. *J Forensic Sci* **2002**; 47(4):773-85.
46. Butler, J. M., Shen, Y. and McCord, B. R. *The development of reduced size STR amplicons as tools for analysis of degraded DNA*. *J Forensic Sci* **2003**; 48(5):1054-64.
47. Chung, D. T., Drabek, J., Opel, K. L., et al. *A study on the effects of degradation and template concentration on the amplification efficiency of the STR Miniplex primer sets*. *J Forensic Sci* **2004**; 49(4):733-40.
48. Hellmann, A., Rohleder, U., Schmitter, H., et al. *STR typing of human telogen hairs--a new approach*. *Int J Legal Med* **2001**; 114(4-5):269-73.
49. Dixon, L. A., Murray, C. M., Archer, E. J., et al. *Validation of a 21-locus autosomal SNP multiplex for forensic identification purposes*. *Forensic Sci Int* **2005**; 154(1):62-77.
50. Coble, M. D. and Butler, J. M. *Characterization of new miniSTR loci to aid analysis of degraded DNA*. *J Forensic Sci* **2005**; 50(1):43-53.
51. Chakraborty, R., Stivers, D. N., Su, B., et al. *The utility of short tandem repeat loci beyond human identification: implications for development of new DNA typing systems*. *Electrophoresis* **1999**; 20(8):1682-96.
52. Gill, P., Werrett, D. J., Budowle, B., et al. *An assessment of whether SNPs will replace STRs in national DNA databases--joint considerations of the DNA working group of the European Network of Forensic Science Institutes (ENFSI) and the Scientific Working Group on DNA Analysis Methods (SWGDM)*. *Sci Justice* **2004**; 44(1):51-3.
53. Sanchez, J. J., Phillips, C., Borsting, C., et al. *A multiplex assay with 52 single nucleotide polymorphisms for human identification*. *Electrophoresis* **2006**; 27(9):1713-24.
54. Gray, M. W. *Origin and evolution of mitochondrial DNA*. *Annu Rev Cell Biol* **1989**; 5:25-50.

55. Hanna, M. G. and Nelson, I. P. *Genetics and molecular pathogenesis of mitochondrial respiratory chain diseases*. Cell Mol Life Sci **1999**; 55(5):691-706.
56. Mitchell, P. D. *Foundations of vectorial metabolism and osmochemistry*. Biosci Rep **2004**; 24(4-5):386-434; discussion 434-5.
57. Prebble, J. *Peter Mitchell and the ox phos wars*. Trends Biochem Sci **2002**; 27(4):209-12.
58. Boyer, P. D. *Toward an adequate scheme for the ATP synthase catalysis*. Biochemistry (Mosc) **2001**; 66(10):1058-66.
59. Service, R. F. *Chemistry prize taps the energy of life*. Science **1997**; 278(5338):579.
60. Stock, D., Gibbons, C., Arechaga, I., et al. *The rotary mechanism of ATP synthase*. Curr Opin Struct Biol **2000**; 10(6):672-9.
61. Anderson, S., Bankier, A. T., Barrell, B. G., et al. *Sequence and organization of the human mitochondrial genome*. Nature **1981**; 290(5806):457-65.
62. Andrews, R. M., Kubacka, I., Chinnery, P. F., et al. *Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA*. Nat Genet **1999**; 23(2):147.
63. Satoh, M. and Kuroiwa, T. *Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell*. Exp Cell Res **1991**; 196(1):137-40.
64. Budowle, B., Wilson, M. R., DiZinno, J. A., et al. *Mitochondrial DNA regions HVI and HVII population data*. Forensic Sci Int **1999**; 103(1):23-35.
65. Giles, R. E., Blanc, H., Cann, H. M., et al. *Maternal inheritance of human mitochondrial DNA*. Proc Natl Acad Sci U S A **1980**; 77(11):6715-9.
66. Brown, W. M., George, M., Jr. and Wilson, A. C. *Rapid evolution of animal mitochondrial DNA*. Proc Natl Acad Sci U S A **1979**; 76(4):1967-71.
67. Richter, C., Park, J. W. and Ames, B. N. *Normal oxidative damage to mitochondrial and nuclear DNA is extensive*. Proc Natl Acad Sci U S A **1988**; 85(17):6465-7.
68. Salas, A., Lareu, M. V. and Carracedo, A. *Heteroplasmy in mtDNA and the weight of evidence in forensic mtDNA analysis: a case report*. Int J Legal Med **2001**; 114(3):186-90.
69. Wallace, D. C. *Mitochondrial diseases in man and mouse*. Science **1999**; 283(5407):1482-8.
70. Allen, M., Engstrom, A. S., Meyers, S., et al. *Mitochondrial DNA sequencing of shed hairs and saliva on robbery caps: sensitivity and matching probabilities*. J Forensic Sci **1998**; 43(3):453-64.
71. Lutz, S., Weisser, H. J., Heizmann, J., et al. *mtDNA as a tool for identification of human remains. Identification using mtDNA*. Int J Legal Med **1996**; 109(4):205-9.
72. Parson, W., Parsons, T. J., Scheithauer, R., et al. *Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: application of*

- mtDNA sequence analysis to a forensic case.* Int J Legal Med **1998**; 111(3):124-32.
73. Szibor, R., Michael, M., Plate, I., et al. *Efficiency of forensic mtDNA analysis. Case examples demonstrating the identification of traces.* Forensic Sci Int **2000**; 113(1-3):71-8.
 74. Wilson, M. R., Polansky, D., Butler, J., et al. *Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts.* Biotechniques **1995**; 18(4):662-9.
 75. Brandstatter, A., Parsons, T. J. and Parson, W. *Rapid screening of mtDNA coding region SNPs for the identification of west European Caucasian haplogroups.* Int J Legal Med **2003**; 117(5):291-8.
 76. Coble, M. D., Just, R. S., O'Callaghan, J. E., et al. *Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians.* Int J Legal Med **2004**; 118(3):137-46.
 77. Herrstadt, C., Elson, J. L., Fahy, E., et al. *Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups.* Am J Hum Genet **2002**; 70(5):1152-71.
 78. Ingman, M., Kaessmann, H., Paabo, S., et al. *Mitochondrial genome variation and the origin of modern humans.* Nature **2000**; 408(6813):708-13.
 79. Gill, P., Whitaker, J., Flaxman, C., et al. *An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA.* Forensic Sci Int **2000**; 112(1):17-40.
 80. Wetton, J. H., Tsang, K. W. and Khan, H. *Inferring the population of origin of DNA evidence within the UK by allele-specific hybridization of Y-SNPs.* Forensic Sci Int **2005**; 152(1):45-53.
 81. Ayub, Q., Mohyuddin, A., Qamar, R., et al. *Identification and characterisation of novel human Y-chromosomal microsatellites from sequence database information.* Nucleic Acids Res **2000**; 28(2):e8.
 82. Kayser, M., Caglia, A., Corach, D., et al. *Evaluation of Y-chromosomal STRs: a multicenter study.* Int J Legal Med **1997**; 110(3):125-33, 141-9.
 83. Krenke, B. E., Viculis, L., Richard, M. L., et al. *Validation of male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex.* Forensic Sci Int **2005**; 151(1):111-24.
 84. Mulero, J. J., Chang, C. W., Calandro, L. M., et al. *Development and validation of the AmpFISTR Yfiler PCR amplification kit: a male specific, single amplification 17 Y-STR multiplex system.* J Forensic Sci **2006**; 51(1):64-75.
 85. Roewer, L., Krawczak, M., Willuweit, S., et al. *Online reference database of European Y-chromosomal short tandem repeat (STR) haplotypes.* Forensic Sci Int **2001**; 118(2-3):106-13.
 86. Sanger, F., Nicklen, S. and Coulson, A. R. *DNA sequencing with chain-terminating inhibitors.* Proc Natl Acad Sci U S A **1977**; 74(12):5463-7.
 87. Rieder, M. J., Taylor, S. L., Tobe, V. O., et al. *Automating the identification of DNA variations using quality-based fluorescence re-*

- sequencing: analysis of the human mitochondrial genome*. Nucleic Acids Res **1998**; 26(4):967-73.
88. Ronaghi, M., Karamohamed, S., Pettersson, B., et al. *Real-time DNA sequencing using detection of pyrophosphate release*. Anal Biochem **1996**; 242(1):84-9.
 89. Ronaghi, M., Uhlen, M. and Nyren, P. *A sequencing method based on real-time pyrophosphate*. Science **1998**; 281(5375):363, 365.
 90. Ahmadian, A., Ehn, M. and Hober, S. *Pyrosequencing: history, biochemistry and future*. Clin Chim Acta **2006**; 363(1-2):83-94.
 91. Langae, T. and Ronaghi, M. *Genetic variation analyses by Pyrosequencing*. Mutat Res **2005**; 573(1-2):96-102.
 92. Andreasson, H., Nilsson, M., Budowle, B., et al. *Quantification of mtDNA mixtures in forensic evidence material using pyrosequencing*. Int J Legal Med **2006**; 120(6):383-90.
 93. Gharizadeh, B., Akhras, M., Nourizad, N., et al. *Methodological improvements of pyrosequencing technology*. J Biotechnol **2006**; 124(3):504-11.
 94. Gharizadeh, B., Herman, Z. S., Eason, R. G., et al. *Large-scale pyrosequencing of synthetic DNA: a comparison with results from Sanger dideoxy sequencing*. Electrophoresis **2006**; 27(15):3042-7.
 95. Ronaghi, M. *Improved performance of pyrosequencing using single-stranded DNA-binding protein*. Anal Biochem **2000**; 286(2):282-8.
 96. Gharizadeh, B., Eriksson, J., Nourizad, N., et al. *Improvements in Pyrosequencing technology by employing Sequenase polymerase*. Anal Biochem **2004**; 330(2):272-80.
 97. Andreasson, H., Asp, A., Alderborn, A., et al. *Mitochondrial sequence analysis for forensic identification using pyrosequencing technology*. Biotechniques **2002**; 32(1):124-6, 128, 130-3.
 98. Andreasson, H., Nilsson, M., Budowle, B., et al. *Nuclear and mitochondrial DNA quantification of various forensic materials*. Forensic Sci Int **2006**; 164(1):56-64.
 99. Nilsson, M., Styrman, H., Andreasson, H., et al. *Sensitive forensic analysis using the Pyrosequencing technology*. International Congress Series **2006**; 1288:625-627.
 100. Margulies, M., Egholm, M., Altman, W. E., et al. *Genome sequencing in microfabricated high-density picolitre reactors*. Nature **2005**; 437(7057):376-80.
 101. Poinar, H. N., Schwarz, C., Qi, J., et al. *Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA*. Science **2006**; 311(5759):392-4.
 102. Stiller, M., Green, R. E., Ronan, M., et al. *Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA*. Proc Natl Acad Sci U S A **2006**; 103(37):13578-84.
 103. Green, R. E., Krause, J., Ptak, S. E., et al. *Analysis of one million base pairs of Neanderthal DNA*. Nature **2006**; 444(7117):330-6.
 104. Noonan, J. P., Coop, G., Kudaravalli, S., et al. *Sequencing and analysis of Neanderthal genomic DNA*. Science **2006**; 314(5802):1113-8.

105. Saiki, R. K., Bugawan, T. L., Horn, G. T., et al. *Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes.* Nature **1986**; 324(6093):163-6.
106. Saiki, R. K., Walsh, P. S., Levenson, C. H., et al. *Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes.* Proc Natl Acad Sci U S A **1989**; 86(16):6230-4.
107. Allen, M., Saldeen, T. and Gyllensten, U. *Allele-specific HLA-DRB1 amplification of forensic evidence samples with mixed genotypes.* Biotechniques **1995**; 19(3):454-63.
108. Calloway, C. D., Reynolds, R. L., Herrin, G. L., Jr., et al. *The frequency of heteroplasmy in the HVII region of mtDNA differs across tissue types and increases with age.* Am J Hum Genet **2000**; 66(4):1384-97.
109. Comas, D., Reynolds, R. and Sajantila, A. *Analysis of mtDNA HVRII in several human populations using an immobilised SSO probe hybridisation assay.* Eur J Hum Genet **1999**; 7(4):459-68.
110. Divne, A. M., Nilsson, M., Calloway, C., et al. *Forensic casework analysis using the HVI/HVII mtDNA linear array assay.* J Forensic Sci **2005**; 50(3):548-54.
111. Gabriel, M. N., Calloway, C. D., Reynolds, R. L., et al. *Identification of human remains by immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariable regions I and II.* Croat Med J **2003**; 44(3):293-8.
112. Reynolds, R., Walker, K., Varlaro, J., et al. *Detection of sequence variation in the HVII region of the human mitochondrial genome in 689 individuals using immobilized sequence-specific oligonucleotide probes.* J Forensic Sci **2000**; 45(6):1210-31.
113. Stoneking, M., Hedgecock, D., Higuchi, R. G., et al. *Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes.* Am J Hum Genet **1991**; 48(2):370-82.
114. Bugawan, T. L., Klitz, W., Alejandrino, M., et al. *The association of specific HLA class I and II alleles with type 1 diabetes among Filipinos.* Tissue Antigens **2002**; 59(6):452-69.
115. Mack, S. J., Bugawan, T. L., Moonsamy, P. V., et al. *Evolution of Pacific/Asian populations inferred from HLA class II allele frequency distributions.* Tissue Antigens **2000**; 55(5):383-400.
116. Erlich, H. A., Opelz, G. and Hansen, J. *HLA DNA typing and transplantation.* Immunity **2001**; 14(4):347-56.
117. Howell, W. M., Jobs, M., Gyllensten, U., et al. *Dynamic allele-specific hybridization. A new method for scoring single nucleotide polymorphisms.* Nat Biotechnol **1999**; 17(1):87-8.
118. Holland, P. M., Abramson, R. D., Watson, R., et al. *Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of *Thermus aquaticus* DNA polymerase.* Proc Natl Acad Sci U S A **1991**; 88(16):7276-80.
119. Livak, K. J., Flood, S. J., Marmaro, J., et al. *Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful*

- for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl* **1995**; 4(6):357-62.
120. Walsh, P. S., Varlaro, J. and Reynolds, R. *A rapid chemiluminescent method for quantitation of human DNA*. *Nucleic Acids Res* **1992**; 20(19):5061-5.
 121. Waye, J. S., Presley, L. A., Budowle, B., et al. *A simple and sensitive method for quantifying human genomic DNA in forensic specimen extracts*. *Biotechniques* **1989**; 7(8):852-5.
 122. Mandrekar, M. N., Erickson, A. M., Kopp, K., et al. *Development of a human DNA quantitation system*. *Croat Med J* **2001**; 42(3):336-9.
 123. Hopwood, A., Oldroyd, N., Fellows, S., et al. *Rapid quantification of DNA samples extracted from buccal scrapes prior to DNA profiling*. *Biotechniques* **1997**; 23(1):18-20.
 124. Diviacco, S., Norio, P., Zentilin, L., et al. *A novel procedure for quantitative polymerase chain reaction by coamplification of competitive templates*. *Gene* **1992**; 122(2):313-20.
 125. Livak, K. J. *Allelic discrimination using fluorogenic probes and the 5' nuclease assay*. *Genet Anal* **1999**; 14(5-6):143-9.
 126. Bar, W., Brinkmann, B., Budowle, B., et al. *DNA Commission of the International Society for Forensic Genetics: guidelines for mitochondrial DNA typing*. *Int J Legal Med* **2000**; 113(4):193-6.
 127. SWGDAM. *Guidelines for Mitochondrial DNA (mtDNA) Nucleotide Sequence Interpretation*. *Forensic Science Communications* **2003**; 5(2).
 128. Budowle, B., Hobson, D. L., Smerick, J. B., et al. *Low copy number - consideration and caution*. in *Twelfth International Symposium on Human Identification* **2001**.
 129. Whitaker, J. P., Cotton, E. A. and Gill, P. *A comparison of the characteristics of profiles produced with the AMPFISTR SGM Plus multiplex system for both standard and low copy number (LCN) STR DNA analysis*. *Forensic Sci Int* **2001**; 123(2-3):215-23.
 130. Lowe, A., Murray, C., Whitaker, J., et al. *The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces*. *Forensic Sci Int* **2002**; 129(1):25.
 131. van Oorschot, R. A. and Jones, M. K. *DNA fingerprints from fingerprints*. *Nature* **1997**; 387(6635):767.
 132. Tully, G., Bar, W., Brinkmann, B., et al. *Considerations by the European DNA profiling (EDNAP) group on the working practices, nomenclature and interpretation of mitochondrial DNA profiles*. *Forensic Sci Int* **2001**; 124(1):83-91.
 133. Wilson, M. R., Allard, M. W., Monson, K., et al. *Recommendations for consistent treatment of length variants in the human mitochondrial DNA control region*. *Forensic Sci Int* **2002**; 129(1):35-42.
 134. Abu Al-Soud, W. and Radstrom, P. *Effects of amplification facilitators on diagnostic PCR in the presence of blood, feces, and meat*. *J Clin Microbiol* **2000**; 38(12):4463-70.

135. Cowell, R. G., Lauritzen, S. L. and Mortera, J. *Identification and separation of DNA mixtures using peak area information*. *Forensic Sci Int* **2006**.
136. Ladd, C., Lee, H. C., Yang, N., et al. *Interpretation of complex forensic DNA mixtures*. *Croat Med J* **2001**; 42(3):244-6.
137. Walker, J. A., Garber, R. K., Hedges, D. J., et al. *Resolution of mixed human DNA samples using mitochondrial DNA sequence variants*. *Anal Biochem* **2004**; 325(1):171-3.
138. Cavelier, L., Jazin, E., Jalonen, P., et al. *MtDNA substitution rate and segregation of heteroplasmy in coding and noncoding regions*. *Hum Genet* **2000**; 107(1):45-50.
139. Lightowers, R. N., Chinnery, P. F., Turnbull, D. M., et al. *Mammalian mitochondrial genetics: heredity, heteroplasmy and disease*. *Trends Genet* **1997**; 13(11):450-5.
140. Wallace, D. C. *Mitochondrial DNA sequence variation in human evolution and disease*. *Proc Natl Acad Sci U S A* **1994**; 91(19):8739-46.
141. Budowle, B., Allard, M. W. and Wilson, M. R. *Critique of interpretation of high levels of heteroplasmy in the human mitochondrial DNA hypervariable region I from hair*. *Forensic Sci Int* **2002**; 126(1):30-3.
142. Tourmen, Y., Baris, O., Dessen, P., et al. *Structure and chromosomal distribution of human mitochondrial pseudogenes*. *Genomics* **2002**; 80(1):71-7.
143. Tsuzuki, T., Nomiya, H., Setoyama, C., et al. *Presence of mitochondrial-DNA-like sequences in the human nuclear DNA*. *Gene* **1983**; 25(2-3):223-9.
144. Mishmar, D., Ruiz-Pesini, E., Brandon, M., et al. *Mitochondrial DNA-like sequences in the nucleus (NUMTs): insights into our African origins and the mechanism of foreign DNA integration*. *Hum Mutat* **2004**; 23(2):125-33.
145. Woischnik, M. and Moraes, C. T. *Pattern of organization of human mitochondrial pseudogenes in the nuclear genome*. *Genome Res* **2002**; 12(6):885-93.
146. Ingman, M. and Gyllensten, U. *mtDB: Human Mitochondrial Genome Database, a resource for population genetics and medical sciences*. *Nucleic Acids Res* **2006**; 34(Database issue):D749-51.
147. Miller, K. W. and Budowle, B. *A compendium of human mitochondrial DNA control region: development of an international standard forensic database*. *Croat Med J* **2001**; 42(3):315-27.
148. Miller, K. W., Dawson, J. L. and Hagelberg, E. *A concordance of nucleotide substitutions in the first and second hypervariable segments of the human mtDNA control region*. *Int J Legal Med* **1996**; 109(3):107-13.
149. Monson, K., Miller, K., Wilson, M., et al. *The mtDNA Population Database: An Integrated Software and Database Resource for Forensic Comparison*. *Forensic Science Communications* **2002**; 4(2).
150. Rohl, A., Brinkmann, B., Forster, L., et al. *An annotated mtDNA database*. *Int J Legal Med* **2001**; 115(1):29-39.

151. Parson, W., Brandstatter, A., Alonso, A., et al. *The EDNAP mitochondrial DNA population database (EMPOP) collaborative exercises: organisation, results and perspectives*. *Forensic Sci Int* **2004**; 139(2-3):215-26.
152. Bandelt, H. J., Salas, A. and Bravi, C. *Problems in FBI mtDNA database*. *Science* **2004**; 305(5689):1402-4.
153. Budowle, B. and Polanskey, D. *FBI mtDNA database: a cogent perspective*. *Science* **2005**; 307(5711):845-7.
154. Budowle, B., Polanskey, D., Allard, M. W., et al. *Addressing the use of phylogenetics for identification of sequences in error in the SWGDAM mitochondrial DNA database*. *J Forensic Sci* **2004**; 49(6):1256-61.
155. Bandelt, H. J., Lahermo, P., Richards, M., et al. *Detecting errors in mtDNA data by phylogenetic analysis*. *Int J Legal Med* **2001**; 115(2):64-9.
156. Just, R. S., Irwin, J. A., O'Callaghan, J. E., et al. *Toward increased utility of mtDNA in forensic identifications*. *Forensic Sci Int* **2004**; 146 Suppl:S147-9.
157. Salas, A., Carracedo, A., Macaulay, V., et al. *A practical guide to mitochondrial DNA error prevention in clinical, forensic, and population genetics*. *Biochem Biophys Res Commun* **2005**; 335(3):891-9.
158. Yao, Y. G., Bravi, C. M. and Bandelt, H. J. *A call for mtDNA data quality control in forensic science*. *Forensic Sci Int* **2004**; 141(1):1-6.
159. Linacre, A. *The UK National DNA Database*. *Lancet* **2003**; 361(9372):1841-2.
160. Martin, P. D., Schmitter, H. and Schneider, P. M. *A brief history of the formation of DNA databases in forensic science within Europe*. *Forensic Sci Int* **2001**; 119(2):225-31.
161. Technology, P. O. o. S. a. *The national DNA database*. postnote **2006**; February(258).
162. Wallace, D. C. *The mitochondrial genome in human adaptive radiation and disease: on the road to therapeutics and performance enhancement*. *Gene* **2005**; 354:169-80.
163. Wallace, D. C. *Mitochondrial defects in neurodegenerative disease*. *Ment Retard Dev Disabil Res Rev* **2001**; 7(3):158-66.
164. Brandon, M. C., Lott, M. T., Nguyen, K. C., et al. *MITOMAP: a human mitochondrial genome database--2004 update*. *Nucleic Acids Res* **2005**; 33 Database Issue:D611-3.
165. De Meirleir, L., Seneca, S., Lissens, W., et al. *Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12*. *J Med Genet* **2004**; 41(2):120-4.
166. McKenzie, M., Liolitsa, D. and Hanna, M. G. *Mitochondrial disease: mutations and mechanisms*. *Neurochem Res* **2004**; 29(3):589-600.
167. Raha, S. and Robinson, B. H. *Mitochondria, oxygen free radicals, disease and ageing*. *Trends Biochem Sci* **2000**; 25(10):502-8.
168. Van Houten, B., Woshner, V. and Santos, J. H. *Role of mitochondrial DNA in toxic responses to oxidative stress*. *DNA Repair (Amst)* **2006**; 5(2):145-52.

169. Corral-Debrinski, M., Horton, T., Lott, M. T., et al. *Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age*. *Nat Genet* **1992**; 2(4):324-9.
170. Lin, M. T. and Beal, M. F. *Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases*. *Nature* **2006**; 443(7113):787-95.
171. Trifunovic, A., Wredenberg, A., Falkenberg, M., et al. *Premature ageing in mice expressing defective mitochondrial DNA polymerase*. *Nature* **2004**; 429(6990):417-23.
172. Feuers, R. J. *The effects of dietary restriction on mitochondrial dysfunction in aging*. *Ann N Y Acad Sci* **1998**; 854:192-201.
173. Harley, C. B., Futcher, A. B. and Greider, C. W. *Telomeres shorten during ageing of human fibroblasts*. *Nature* **1990**; 345(6274):458-60.
174. Cooper, A. and Poinar, H. N. *Ancient DNA: do it right or not at all*. *Science* **2000**; 289(5482):1139.
175. Gilbert, M. T., Bandelt, H. J., Hofreiter, M., et al. *Assessing ancient DNA studies*. *Trends Ecol Evol* **2005**; 20(10):541-4.
176. Hofreiter, M., Serre, D., Poinar, H. N., et al. *Ancient DNA*. *Nat Rev Genet* **2001**; 2(5):353-9.
177. Paabo, S., Poinar, H., Serre, D., et al. *Genetic analyses from ancient DNA*. *Annu Rev Genet* **2004**; 38:645-79.
178. Handt, O., Richards, M., Trommsdorff, M., et al. *Molecular genetic analyses of the Tyrolean Ice Man*. *Science* **1994**; 264(5166):1775-8.
179. Krause, J., Dear, P. H., Pollack, J. L., et al. *Multiplex amplification of the mammoth mitochondrial genome and the evolution of Elephantidae*. *Nature* **2006**; 439(7077):724-7.
180. Rogaev, E. I., Moliaka, Y. K., Malyarchuk, B. A., et al. *Complete mitochondrial genome and phylogeny of Pleistocene Mammoth *Mammuthus primigenius**. *PLoS Biol* **2006**; 4(3):e73.
181. Vernesi, C., Di Benedetto, G., Caramelli, D., et al. *Genetic characterization of the body attributed to the evangelist Luke*. *Proc Natl Acad Sci U S A* **2001**; 98(23):13460-3.
182. Jehaes, E., Decorte, R., Peneau, A., et al. *Mitochondrial DNA analysis on remains of a putative son of Louis XVI, King of France and Marie-Antoinette*. *Eur J Hum Genet* **1998**; 6(4):383-95.
183. Stone, A. C., Starrs, J. E. and Stoneking, M. *Mitochondrial DNA analysis of the presumptive remains of Jesse James*. *J Forensic Sci* **2001**; 46(1):173-6.
184. Andreasson, H., Gyllensten, U. and Allen, M. *Real-time DNA quantification of nuclear and mitochondrial DNA in forensic analysis*. *Biotechniques* **2002**; 33(2):402-4, 407-11.
185. Linch, C. A., Smith, S. L. and Prahlow, J. A. *Evaluation of the human hair root for DNA typing subsequent to microscopic comparison*. *J Forensic Sci* **1998**; 43(2):305-14.
186. Divne, A. M. and Allen, M. *A DNA microarray system for forensic SNP analysis*. *Forensic Sci Int* **2005**; 154(2-3):111-21.

187. Quintans, B., Alvarez-Iglesias, V., Salas, A., et al. *Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequencing*. *Forensic Sci Int* **2004**; 140(2-3):251-7.
188. Vallone, P. M., Just, R. S., Coble, M. D., et al. *A multiplex allele-specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome*. *Int J Legal Med* **2004**; 118(3):147-57.
189. Andreasson, H., Nilsson, M., Styrman, H., et al. *Forensic mitochondrial coding region analysis for increased discrimination using pyrosequencing technology*. *Forensic Science International: Genetics* **In Press (2007)**; doi:10.1016/j.fsigen.2006.10.002.
190. Brandstatter, A., Salas, A., Niederstatter, H., et al. *Dissection of mitochondrial superhaplogroup H using coding region SNPs*. *Electrophoresis* **2006**; 27(13):2541-50.
191. Grignani, P., Peloso, G., Achilli, A., et al. *Subtyping mtDNA haplogroup H by SNaPshot minisequencing and its application in forensic individual identification*. *Int J Legal Med* **2005**:1-6.
192. Pereira, L., Richards, M., Goios, A., et al. *Evaluating the forensic informativeness of mtDNA haplogroup H sub-typing on a Eurasian scale*. *Forensic Sci Int* **2005**.
193. LaBerge, G. S., Shelton, R. J. and Danielson, P. B. *Forensic utility of mitochondrial DNA analysis based on denaturing high-performance liquid chromatography*. *Croat Med J* **2003**; 44(3):281-8.
194. Lutz-Bonengel, S., Sanger, T., Pollak, S., et al. *Different methods to determine length heteroplasmy within the mitochondrial control region*. *Int J Legal Med* **2004**; 118(5):274-81.
195. Niederstatter, H., Coble, M. D., Grubwieser, P., et al. *Characterization of mtDNA SNP typing and mixture ratio assessment with simultaneous real-time PCR quantification of both allelic states*. *Int J Legal Med* **2006**; 120(1):18-23.
196. Juusola, J. and Ballantyne, J. *Multiplex mRNA profiling for the identification of body fluids*. *Forensic Sci Int* **2005**; 152(1):1-12.
197. Yang, N., Li, H., Criswell, L. A., et al. *Examination of ancestry and ethnic affiliation using highly informative diallelic DNA markers: application to diverse and admixed populations and implications for clinical epidemiology and forensic medicine*. *Hum Genet* **2005**; 118(3-4):382-92.
198. Coussens, A. K. and van Daal, A. *Linkage disequilibrium analysis identifies an FGFR1 haplotype-tag SNP associated with normal variation in craniofacial shape*. *Genomics* **2005**; 85(5):563-73.
199. Graf, J., Hodgson, R. and van Daal, A. *Single nucleotide polymorphisms in the MATP gene are associated with normal human pigmentation variation*. *Hum Mutat* **2005**; 25(3):278-84.
200. Sturm, R. A. and Frudakis, T. N. *Eye colour: portals into pigmentation genes and ancestry*. *Trends Genet* **2004**; 20(8):327-32.

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