

Profiling in Dental Forensics Odontology - Part II: DNA Profiling Systems and Future Directions

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Abstract

In the first part of this DNA profile series, an overview of the importance of this method in forensics was given along with a brief description of the DNA isolation methods. This second section provides an account of the few profiling programs currently available and the future scope of these approaches to forensic odontology.

Keywords: DNA profiling; mtDNA; Dental DNA; DNA Analysis

Introduction

During DNA profiles, cells are collected and broken down to reach their DNA. Forensic scientists then copy the regions of DNA of their interest and estimate the length of the sequence in multiple loci. The length of the direct sequence of repeats serves as the marker of the DNA profiles because the length of the repeat is sufficient to distinguish between individuals. Forensic scientists prefer to analyze a small set of markers, rarely more than one locus per chromosome. Prior to the increased sensitivity provided by PCR, large samples such as stains of blood measuring an inch or a quarter were needed to obtain sufficient DNA to form a profile. With the advent of PCR and subsequent development, it is now easier to obtain DNA profiles in very small quantities. samples.¹

DNA Typing Systems

(DNA profiling) uses a variety of DNA typing systems including: Restriction Fragment Length Polymorphism (RFLP), Short Tandem Repeat (STR) typing, Mitochondrial DNA (mtDNA) analysis, Y chromosome analysis, X-chromosome STR typing, Single Nucleotide Polymorphism (SNP) typing and gender typing.

Restriction Fragment Length Polymorphism (RFLP)

Typing RFLP is one of the oldest DNA typing methods that is no longer in use. It is used to analyze the variable lengths of DNA fragments that result from the digestion of a DNA sample with a special type of inhibitory enzyme called “restriction endonuclease” that binds DNA to a specific sequence pattern known as the endonuclease

recognition site. With the development of new, more efficient DNA analysis techniques, RFLP is not used as it was before, because it requires a large amount of DNA, cannot be processed by samples that are damaged by natural factors and also takes a long time to obtain results.^{2,3}

Short Tandem Repeats (STRs)

Its defined as short DNA strips that are replicated in various parts of the human genome and this technology is used to examine specific regions (loci) within nuclear DNA. Each person has a STR defect from the father and some from the mother and yet no one has the same STR as those of a single parent. Variation of human STRs provides a patent for science and therefore aids in formal identification and paternal testing. As there are so many variations in STR the opportunity to match two different people is very remote.^{4,6}

CODIS CODIS stands for Combined DNA Index System. It was founded and funded by the Federal Bureau of Investigation (FBI) which has become the core of the national DNA database. Designed specifically to enable public forensic DNA laboratories to create searchable DNA database of authorized DNA profiles. The CODIS software allows laboratories throughout the United States (US) to share and compare DNA data. In addition, it provides centralized database of DNA profiles from all user laboratories. Surprisingly, two people will have the same 13-loci DNA profile for about one billion. The 13 locations of CODIS are TH01, TPOX, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11. The amelogenin tag for sex typing is usually inserted into STR multiplexes that include 13 core STR loci. The United States maintains the largest DNA database in the world: The Combined DNA Index System, with more than 60 million records since 2007.⁷⁻⁹ Bone and dental use in STR analysis can be used to identify bodies in major disasters and fossils.¹⁰⁻¹² Separated DNA and fossils can be included in the STR analysis and although the DNA present in these fossils appears to be highly degraded, they were better preserved in the tooth than in bone samples.¹³ Correct sample of body parts is important to get good quality DNA for analysis. Milos A et al reported that high success rates were observed with samples from strong cortical of weight-bearing-lower limb bones (femur 86.9%) and strong teeth also showed high success rates (82.7% teeth).¹⁴ Commercial kits are available as AmpFSTR Profiler Kit, AmpFSTR Profiler Plus Kit, AmpFSTRCOfiler Kit, and PowerPlex¹⁶ system, that are the most sensitive multilocus

STR enhancement programs, which can be used effectively to obtain the multilocus profile of older teeth and bone samples but have a small amount of human DNA or even invisible human DNA.¹⁵

Mitochondrial DNA (mtDNA)

The long intervals between death and tissue testing make it difficult to identify genes and sometimes only bones and teeth can be found for analysis. Several researchers have commented on the division of nuclear DNA into these substances, but all have concluded that DNA is highly degraded.¹⁶ Teeth provide an excellent source of molecular weight mtDNA that can be extremely valuable in the forensic investigation of decaying human remains.¹⁷ Mitochondrial DNA sequencing offers several unique benefits of identifying human remains.^{18,19} Its analysis uses DNA extracted from the mitochondrion which is another cell organelle. Various biological samples consisting of hair, bones, and tooth that lack nucleated cell material may be analyzed with mtDNA and could be very useful in fixing antique unsolved forensic cases.^{20,21} mtDNA is a effective device for forensic identification as it possesses excessive reproduction variety, maternal inheritance, and excessive diploma of collection variability. each offspring have the equal mitochondrial DNA as their mothers for the reason that mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by using father's sperm.

In a study involving missing individuals, comparing the mtDNA profile of an unknown fossil with the profile of a potential maternal relative may be important.^{22,23} Strategies of mtDNA evaluation Forensic analysis of mitochondrial D-loop sequences, involving the usage of Sanger sequencing or SNP detection via mini sequencing is nicely established. However, pyrosequencing has turn out to be an critical opportunity as it enables complete evaluation and the quantification of person mtDNAs in samples originating from more than one person.²⁴ Trace amounts of DNA or big degradation can result in failure of STR analysis.

In these cases the DNA typing of the mitochondrial displacement loop (D-loop) is usually the investigator's choice as most of the human mtDNA variants are found within the D-loop and can be useful in discriminating between unrelated individuals.²⁵ The HV I/HV II mtDNA linear array assay, decreases sequencing efforts appreciably and thereby lowering the value and time in forensic evaluation.²⁶

Y-Chromosome Analysis

DNA-polymorphisms in the human Y chromosome are important tools for understanding evolution and human migration.^{27,28} The Y chromosome is especially useful in tracing male relationships or analyzing biological evidence involving multiple male donors as the Y chromosome is transmitted directly from father to son. The maximum length of the human Y chromosome is achieved as a single block in the connection from father to male offspring as a haploid business. Therefore, the Y chromosome is a valuable record of all the changes that took place in the ranks of men throughout evolution and migration. Y chromosomal DNA variants have therefore been used primarily for research on human origin and for scientific purposes or for biological analysis whilst a male suspect is involved.

Y-STR analysis

In the analysis of spots of sexual assault involving both males and females and when the first one is at a low level of concentration, Y-STR analysis can be particularly helpful in locating a fraction of male DNA.³⁰⁻³² Haploidy and patrilineal inheritance make it difficult to interpret the same Y STR, because male relatives share several generations with the same Y-STR profile. Since the relatives of the fathers often live in the area and culture in the area of their ancestors, the analysis of the Y chromosome has the potential to draw conclusions about the human origin of a given DNA profile.^{33,34} DNA separated from the teeth and bones of World War II. II fossils of the victims were used for identification using the combined Y chromosome (STR) and MiniSTR method of Y STR analysis. The Y-PLEX 12 system allows simultaneous enlargement of eleven polymorphic short tandem repeat (STR) loci, which reside in the Y chromosome and amelogenin that can be used in cases of male genetic identification. Amelogenin provides sex-detecting results and acts as an internal control of PCR.³⁸

X-chromosome STR

Specific X chromosome X is used in the identification and genomic studies of various species on Earth.³⁹⁻⁴¹ From X-chromosome size STR alleles are small, usually comprising 100-350 nucleotides, relatively comparable in magnification to high sensitivity. Many X-linked microsatellites have been tested but further research is needed to determine specific demographics.⁴³

Nucleotide polymorphism (SNPs)

One nucleotide polymorphisms (SNPs) are DNA sequences that occur when a single nucleotide (A, T, C, or G) in the genome series is modified. For example the SNP may convert DNA sequences AAGGCTAA to ATGGCTAA.⁴⁴ SNPs are rising as new markers of interest to the forensic medication due to their small amplicon length that is useful in analysing degraded samples, decrease mutation rate compared with STRs, amenable to excessive during evaluation (automation), abundant within the human genome, can offer precise facts approximately about ancestry, genealogy, evolution, or phenotype, and determine gender.^{45,46} F forensic analytical SNPs can be classified into four categories such as SNPs identity verification., genetic SNPs, ancestral SNPs, and phenotype pedagogical SNPs.⁴⁷ In recent years, SNPs have been widely used in a variety of applications, including medical diagnostics, genetics, and personal identity testing.^{48,49} There all DNA typing systems failed to identify a single person, SNPs have the ability to provide a straightforward solution and this was proved when forensic scientists used SNP technology continuously to identify several victims 9/11.⁵⁰ Pakstis AJ et al⁵¹ recently developed an active tool worldwide 92 individual SNPs for individual identification (IISNPs) and the lowest probability of any two unrelated individuals from anywhere in the world with similar genetic makeup.

The limitations of SNPs include such a lack of a well-established core loci, as well as a requirement for extensive multiplexing testing. SNPs are unlikely to replace the main STR currently used in the national DNA database, links, structure due to low conversion rates, multiple typing platforms making it difficult for universal SNP selection.⁴⁵ Efforts are being made to investigate whether or not it may replace STR but nevertheless SNPs are the DNA technology of the future. Gender typing The tooth proteins needed to develop normal dental enamel coded amelogenin genes.

These genes are part of a small group of genes that are active in both sex chromosomes.⁵² The amelogenin gene is a single copy, the homologues found in Xp22.1-Xp22.3 and Yp 11.2.⁵³ Length Differences in XeY, homologous amelogenin gene (AMELX and AMELY), is used to identify sex and is an integral part of many PCR kits currently used to process DNA.^{54,55} Dental pulp is an important source of DNA for determining sex.⁵⁶ Komuro T et al⁵⁷ identified sex in DNA of dental pulp by analysis of X and Y locus peaks by capillary gel electrophoresis (CGE).

Many commercial kits are available as a

geneprint sex determination that gives better results.⁵⁸ Future directions A short PMI (post-mortem interval) and / or dry area can reap DNA preservation in the pulp, while a longer PMI and / or wet environment would increase reliance on hard tooth tissues for DNA. Despite the increasing use of dental tissue in forensic investigations, little reliable information has been obtained on the decay processes of these mineral-rich tissues, in the DNA sequence following post-mortem diagenesis, or in the results of various sampling techniques. Further in-depth research is needed to understand the interaction between tooth minerals and DNA and how these mutations change at the time of death.

Proposed methods of DNA retention in the teeth, which have not been thoroughly investigated include: encapsulation inside the pulp chamber, binding to minerals and binding to collagen. This has important implications for selecting effective samples and extraction methods. Examination of post-mortem changes in the teeth during the period covered by the forensic examination may also be very important. This information will allow for the most appropriate tissue selection for DNA extraction, as well as more informed selection of the technique used to release DNA, which increases the efficiency of the extraction process. Effective ways to eliminate pollution can also be determined.

Conclusion

The use of DNA technology has revolutionized forensic diagnostic procedures since its inception 25 years ago. Teeth are an excellent source of both nuclear and mitochondrial DNA, and have been used successfully in the official identification of endangered human fossils. However, the techniques currently used in forensic research to obtain DNA from these tissues do not show an understanding of the alternative way to make teeth. In fact, small targeted sampling of certain dental tissues is rarely performed, and complete dental extraction is performed routinely. It is clear that a complete understanding of tooth structure, as well as the process of diagenesis in teeth, is important in determining the location of DNA in post-mortem teeth.

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