

Applications of DNA Profiling in Dental Forensics - Part I Background & Overview

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Abstract

Teeth are a valuable source of DNA and resistant to adverse conditions such as incineration, immersion, trauma, mutilation, decomposition when compared to other parts of the body that are destroyed or degraded in mass disasters, so they are highly useful in forensic investigations. DNA profiling gives an exact identification of culprits in crime scene investigations, identification of an individual in mass disasters as well as solving paternity issues. It also provides information regarding the physical characteristics, ethnicity and sex determination. The recent advancements in the technology of DNA research have revolutionized the field of forensic odontology and this paper provides an insight into the recent concepts of DNA profiling in forensic dentistry.

Keywords: DNA profiling; mtDNA; Dental DNA; DNA analysis.

Introduction

The world has experienced many mass disasters like acts of terrorism, bombings, earthquakes, tsunami and transportation mishaps in the recent times¹. Disaster victim identification relies on the efforts of police, dentists and pathologists^{2,4}. Because of the resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation and decomposition, teeth represent an excellent source of DNA material⁵⁻⁷. When the conventional dental identification methods fail, this biological material can provide the necessary link to prove identity⁸⁻¹⁰.

By drawing together the current knowledge of tooth structure and post-mortem diagnosis of DNA in tooth tissues, this paper offers optimization of tooth selection and targeted sampling to maximize successful outcomes of DNA extraction and profiling.

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Role of DNA in Identification

Any type of organism can be identified by examination of DNA sequences unique to that species. Every cell of an individual carries a copy of the DNA¹¹. This uniqueness is due to the intron regions of DNA which contain sequences that are 20e100bp in length that are repeated at different locations (loci) along the chromosome like AGACTAGACATT – AGATTAGGCATT which are called sequence polymorphism¹². The length polymorphism like (-AATG)(AATG)(AATG) (3 repeats) and (AATG)(AATG) (2 repeats) are termed as Short tandem repeats (STRs) which are used in forensic identification^{16,17}.

History

The Discovery of DNA

DNA, a double helix molecule carrying genetic material which determines the makeup of all living cells from one generation to the other was discovered by James Watson and Francis Crick in 1953¹⁸. They presented the structure of the DNA helix for the first time and shared the Nobel Prize with Maurice Wilkins, nine years later in 1962, for solving one of the most important biological riddles¹⁹.



Forensic DNA Analysis

In 1985 an English geneticist named Dr. Alec Jeffreys first described 'DNA fingerprinting' or now known as DNA typing (profiling)²⁰. He discovered that DNA sequence in certain regions were repeated and were different in each individual²¹. Jeffreys developed a technique to examine the length variation of these DNA repeat sequences which had the ability to perform human identity tests²². These DNA repeat regions are called as VNTRs (variable number tandem repeats) and was first used to solve

an English immigration case and shortly thereafter to find out the culprit in a double homicide case and since then, human identification using DNA profiling methods has been widespread²³.

Evetts and Buckleton advocated a change to DNA profiling that has been largely accepted. DNA profiling made the system more sensitive, more reproducible, amenable to computer databasing, and soon became the standard forensic DNA system used in criminal case work as well as paternity testing worldwide²⁴.

Guidelines for Obtaining Dental DNA

Teeth and bones are the only sources of DNA material which is available for the identification of fragmented or degraded human body parts. The location of teeth in the jawbone and their unique composition provide additional protection to DNA as compared to bones making them a superior source of DNA in many cases.

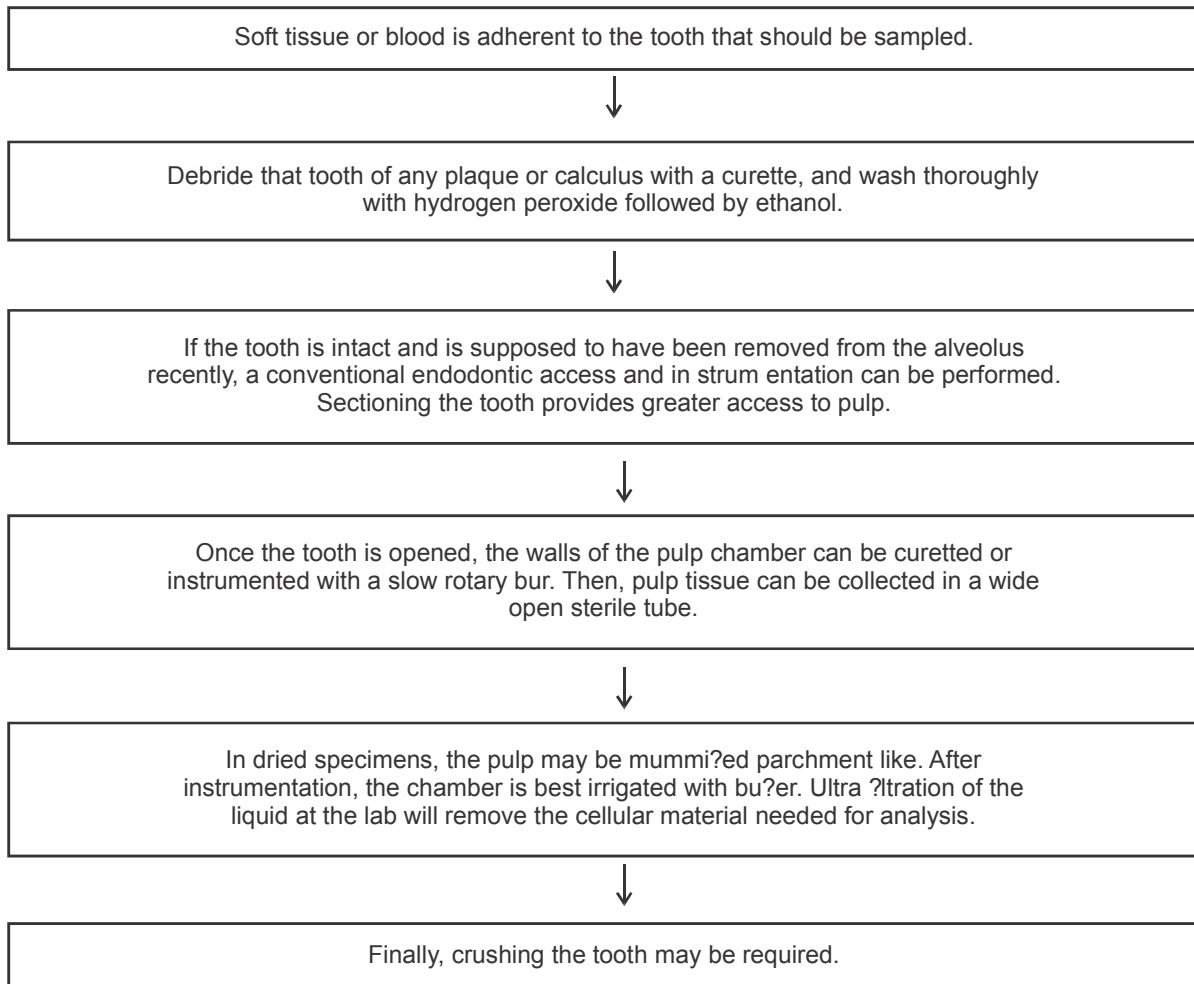


Fig. 1: Guidelines for Obtaining Dental DNA

DNA Teeth and Saliva

DNA can be isolated from various sources, as long as they contain nucleated cells.

Collection of Samples

Teeth are the good source of genetic information. Nucleated cells can be isolated from the surrounding bone, periodontal fibers, and blood. However, the

chance of contamination or degradation is very large, except in the pulp. DNA material from teeth can be achieved by crushing.

Conventional Endodontic Access

It is difficult to obtain enough DNA through conventional endodontic access. In addition, the occlusal morphology and the restorations are damaged¹³.

Vertical Splitting

A lot of pulp tissue can be obtained with the vertical splitting method, although the restorations and the tooth are damaged¹⁴.

Horizontal Section

It is the technique by which the tooth is cut at the cervix region. As the crown of teeth remains intact, the access of the pulp and the roots is sufficient.

Cryogenic Grinding

The tooth is kept in an electromagnetic chamber after freezing it in liquid nitrogen. By alternating the magnetic fields, the tooth is grinded into a fine powder¹⁵.

More amount of DNA can be obtained by crushing method rather than just sectioning of teeth as the chance of DNA damage can be minimized considering the sensitivity of PCR¹¹. (Figure.2)

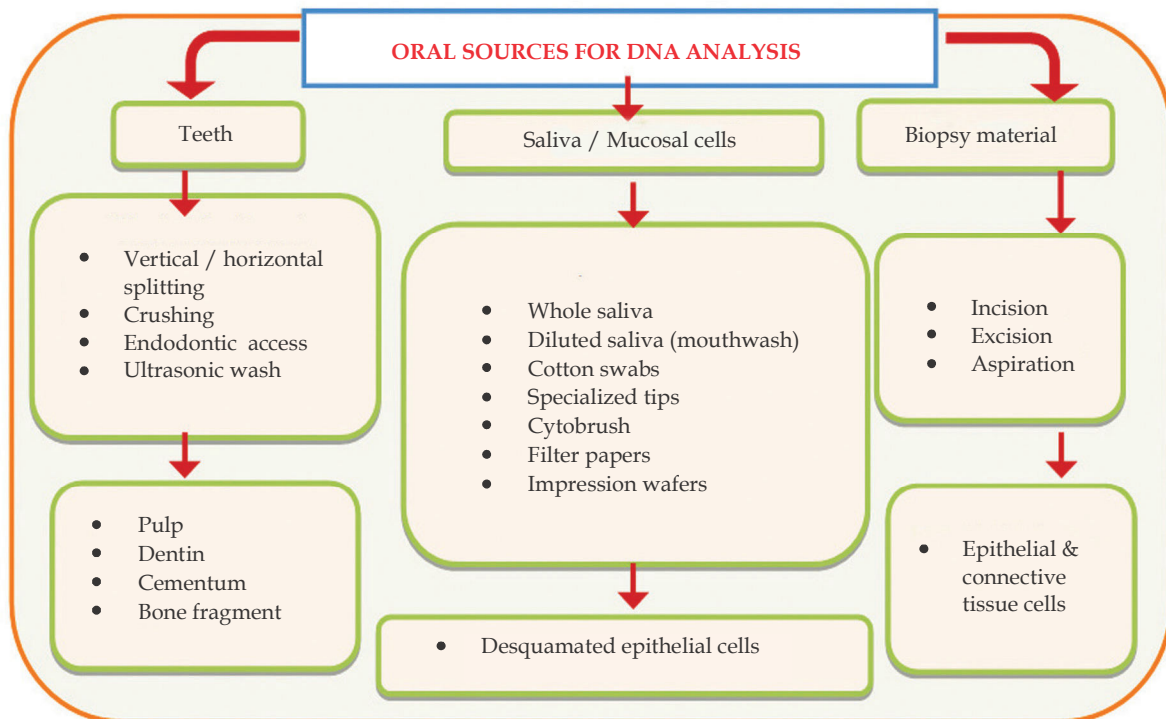


Fig. 2: Oral Sources for DNA Analysis

Factors Influencing DNA Content

Some of the factors indicated to have an effect on DNA content include tooth type, chronological age of the donor, and health status of the tooth. Each of these factors will influence the relative proportions of DNA present in the crown and root, and in the pulp, dentine and cementum.

Tooth Type

It has also been shown that more DNA is retrieved from multi-rooted teeth than single rooted teeth. In the absence of molars, premolars would be expected to have more cellular cementum than anterior teeth, but canines would have a larger pulp volume.

Chronological Age

Advancing age leads to a decrease in DNA content and a change in the distribution of DNA throughout the tooth. Whilst in mature individuals molars would still be the teeth of choice, factors such as the extent of tooth wear and the abundance of cementum should be considered when selecting a sample. Sample choice may be limited in older individuals due to increased exposure to dental disease, and dental treatment and increased tooth loss. Dental diseases not only reduce the amount of DNA available but also increase the potential for contamination.

Teeth and DNA Analysis

Ancient DNA (aDNA) analysis can be carried out through extraction of the tiny amount of DNA remaining in samples that are hundreds to tens of thousands of years old²⁵. Teeth are resistant to adverse conditions degrading the DNA, such as humidity, high temperature, and the microbial action^{26,28}. In the tooth, dentin and pulp are rich sources of DNA which can be successfully extracted^{28,29}. Results of a study demonstrated that sufficient quantity of DNA can be extracted from the crown body, root body, and root tip. However, the root body is the region which yields highest quantities of DNA³⁰. Not only the quantity of DNA available for the laboratory is important, but also the quality and purity. Furthermore, an abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis^{31,32}.

There are various steps in DNA analysis- Extraction of the dental DNA, quantitation followed by DNA amplification and separation and finally DNA analysis and interpretation. (Figure.3)

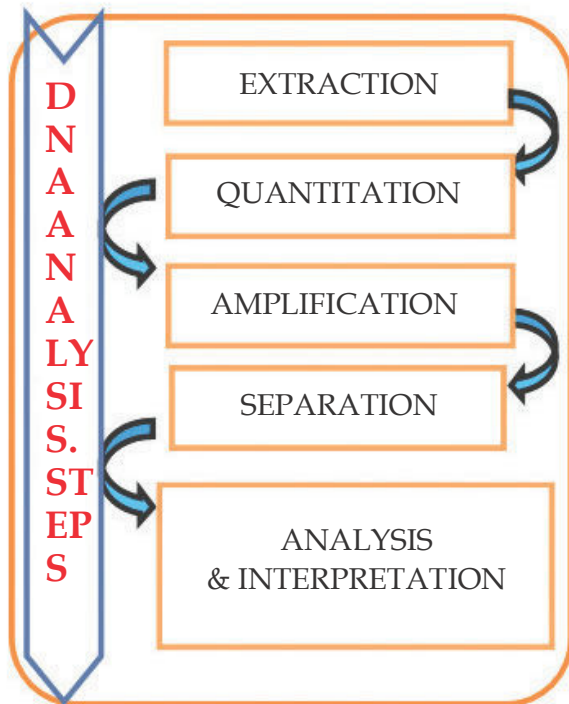


Fig. 3: Basic Steps in DNA Analysis

DNA Isolation Methods

Forensic DNA analysis can be increasingly problematic since samples from the scene of crime or a mass disaster may contain only minute amounts of DNA, which may include polymerase chain reaction (PCR)-inhibitors. Efficient DNA extraction

procedures as well as accurate DNA quantification methods are critical steps involved in the process of successful DNA analysis of such samples³⁷.

Organic Extraction (Phenol-Chloroform Method)

This method of isolation yields high quality DNA, it has many disadvantages like it is very laborious, time consuming, handling of dangerous organic solvents and can only be done if abundance of sample is available. Due to these setbacks, the phenol/chloroform method has been superseded by many other techniques which have made it irrelevant at present^{37,38}.

Silica Based DNA Extraction Methods

Silica based methods are used for isolation of DNA from ancient bones and teeth (aDNA). The silica-based extraction method showed better results in nuclear STR typing from degraded bone samples than a commonly used phenol/chloroform method³⁹. Digesting of the bone powders with proteinase K, and then extracting purified DNA directly using silica-based spin columns (QIAquick, QIAGEN) is also an efficient method of DNA isolation⁴⁰. Recovery of PCR-amplifiable DNA from ancient bone and teeth specimens can be maximised by a combination of DNA extraction from bone powder using a buffer consisting solely of EDTA and proteinase K, and purification of the DNA by binding to silica⁴¹. Silica-based aDNA extracts using ion-exchange columns considerably improved PCR amplification and can be useful in poorly preserved, PCR-resistant, ancient samples⁴². A combination of total demineralization and ion-exchange columns increases approximately three times higher DNA recovery from old bone compared to incomplete demineralization method^{43,44}.

Chelex 100

The procedures are simple, rapid, involve no organic solvents and do not require multiple tube transfers for most types of samples⁴⁵. The extraction of DNA from dental pulp using this method is reported to be efficient compared to proteinase K and phenol-chloroform extraction. Chelex 100-based DNA extraction, amplification, and typing are possible in incinerated teeth⁴⁶.

Commercial DNA Extraction Kits

The PrepFiler Forensic DNA extraction kit enables isolation of genomic DNA from a variety of biological samples. The kit facilitates reversible binding of DNA with magnetic particles resulting in high DNA recovery from samples with very low and high quantities of biological materials example

saliva on swabs^{47,48}.

DNA Amplification Methods

The Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a method widely used to rapidly make millions to billions of copies of a specific DNA and amplify it to a large enough amount to study in detail⁴⁹. Polymerase Chain Reaction (PCR) was developed in the year 1983 by Kary Mullis for which he was awarded Nobel prize in chemistry in 1993^{50,51}. The drawbacks of PCR is that Quantification is exceedingly difficult⁵².

Real Time Polymerase Chain Reaction

Real time PCR is refinement of the old PCR technique and is the most powerful tool for DNA amplification. Some of the limitations of the old PCR were resolved in 1992 by the development of real-time PCR. It was developed by Higuchi et al⁵³. Typical uses of real-time PCR includes pathogen detection, gene expression analysis, single nucleotide polymorphism (SNP) analysis, analysis of chromosome aberrations, and most recently the protein detection as well⁵²⁻⁵⁵.

AmpFISTR MiniFiler and AmpFISTR Identifier PCR Amplification Kits

The AmpFISTR MiniFiler polymerase chain reaction amplification kit, developed and supplied by Applied Biosystems, complements the AmpFISTR Identifier polymerase chain reaction amplification kit⁵⁶.

Conclusion

Teeth are an excellent source of both nuclear and mitochondrial DNA, and have been successfully used in the forensic identification of compromised human skeletal remains. A comprehensive understanding of tooth structure and composition, as well as the process of diagenesis in teeth, is crucial for determining the location of DNA in post-mortem teeth. A detailed description of DNA profiling and its various methods will be laid out in the next part of this paper.

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