DNA from teeth for use in forensic identification

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"*Under most conditions occurring in nature, the teeth are the least destructible part of the body and they may readily survive all these changes... Fire, putrefaction or prolonged immersion in water.*"

- Wittaker and MacDonald, 1989

Preface

We have chosen to write our master thesis on the subject of forensic dentistry as we both share an interest in forensics and wanted to learn more about this branch of science. The use of dental DNA in forensics in human identification cases is still a relatively new method and it is not well understood by our fellow students and colleagues. While searching for the literature we found a lot of the papers to be difficult and complicated to read. Our idea for this project was therefore to write an explanatory review about this topic making it more approachable for the average reader. We also wanted to raise more awareness to another role dentistry may play in forensic identification.

As our project is mainly a literature study, we explored a great number of publications and used them to summarize the present use of dental DNA in forensics. In addition to this, we got the opportunity to follow the protocol for the DNA extraction, quantification and establishing of the DNA profile presented on one of the authors' 3rd molars (Anna Zajkowska).

We would like to give a special thanks to Marguerethe Stenersen at the Institute of Forensic Medicine at Oslo University Hospital for making the laboratory test in this study possible, and especially to Dorota Perchla who guided us through the process. Great thanks are also due to associate professor Sigrid I. Kvaal for advice in the initial phase of the project, and to Maria Pain, Scientist in Microbiology at Nordic Institute of Dental Materials (NIOM) for valuable comments on our manuscript. Finally, we wish to thank our supervisor, major Simen E. Kopperud from the Norwegian Armed Forces for giving us constructive feedback and advice during our work with this master's thesis.

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Introduction

Forensic dentistry is a branch of forensic sciences that uses the knowledge of dental professionals in identification cases. The identification of human remains is primarily based on comparing ante mortem (AM) data with the post mortem (PM) reports. A triad of reasons are mentioned when justifying identification of a deceased individual: social, legal, and forensic reasons (Adams, 2014, p 65). Establishing identity of an individual plays an important role in providing closure for surviving relatives both in terms of progressing through the grieving process, but also when it comes to settling business and personal affairs. Criminal investigation and potential prosecution in homicide cases may also not be possible to proceed if the victim remains unidentified ("American Board of Forensic Odontology").

In 1984 INTERPOL published the first Disaster Victim Identification (DVI) Guide. Over the years, the experience gained by the past and present international community of DVI has been considered, and the guide has been updated every five years since (INTERPOL, 2018, p. 6, 7). Generally speaking, there are four primary methods used to identify deceased human remains: 1) fingerprints (ridgeology), 2) DNA profiling, 3) analyses of physical indicators as tattoos, scars and surgical implants and 4) dental examinations. The method or methods of choice will often be correlated to the degree of decomposition or destruction of the body, the amount and quality of the AM records and the preference of the investigating instances.

Dental identification is, as mentioned above, based on comparison of AM and PM dental characteristics of an individual given that dental records (i.e., written records, digital images, study casts, photographs, and supportive radiographs) are available. The basis of the dental identification is the fact that two oral cavities are not alike, and the teeth are unique to an individual (Krishan, 2015). As teeth are the hardest and most robust tissue of the human body, they are often resistant to decomposition even in severe exposure to the elements, such as major accidents, crime, burning damages, aging, burials, or other (Krishan, 2015). In literature, dental analysis is described as a relatively quick, cheap, and easy method of identification.

In Norway, most ID-cases are today solved by using DNA fingerprint analyses. Nevertheless, the teeth may still play an important role. In several cases, the remains are so degraded or fragmented, that teeth and bones are the only sources of the genetic material. Although DNA

profiling is the main forensic analysis technique used in human identification cases, it can be challenging to obtain DNA in a sufficient quality and quantity. Sometimes the DNA profile achieved may even not be informative enough for use in forensic identification (Heatfield, 2021). Because the teeth are located in the alveolar bone and covered by soft tissue in the lips and cheeks, their DNA is additionally protected compared with other tissues. That make them an even more preferred source of the genetic material than bones in very decomposed remains **(**Higgins, 2013**)**.

The principle of DNA fingerprinting analysis was first introduced by Jeffreys in 1985, and was based on simple minisatellite hybridization (Ziętkiewicz, 2012). Shortly after the first successful DNA profiling, the PCR technique was described by Saiki *et* al (1985) allowing identification based on less material of lower quality. Since then, molecular approaches to forensic science have undergone rapid evolution and became accepted as a legitimate tool for identification. After the evidence is collected from the site of investigation, the DNA is isolated, cut at specific sites with restriction enzymes and amplified using the PCR principle. As small quantity as 125 pg of good-quality DNA (corresponding to only 15-20 human cells) is sufficient to carry on with molecular analyses (Latham, 2019). DNA fingerprinting usually targets short non-coding sequences in the genome – *short tandem repeats* (STRs). Up to 30 STRs can be detected, sequenced, and arranged by their molecular sizes using capillary electrophoresis (Roewer, 2013). Each STR is highly polymorphic and will be slightly different for every individual. Therefore, each DNA profile obtained from the deceased person's remains will make a unique pattern of STRs making it possible to match it with DNA profile produced from AM samples (Adams, 2014, p 108-109).

Aim

This master thesis aims to be a description of the use of teeth for DNA analysis for use in human identification. We have used literature from searches in the PubMed database with the keywords "forensic dentistry", "DNA", "teeth", and "PCR". In addition, we also used textbooks in forensic odontology and biochemistry. We have chosen to study articles that are relevant for the practical experiment done at the laboratory at Institute of Forensic Medicine at Oslo University Hospital and topics related to it.

Structure of DNA

DNA or deoxyribonucleic acid contains our genetic code, it exists in eukaryotic cells that contain a nucleus and in the mitochondria of the cell. The DNA molecule is coiled tightly together around proteins called histones to form a bigger molecule called a chromosome. Humans have in total 23 pairs of chromosomes. 22 of these look the same in both sexes, but the 23rd pair is the sex chromosome that will differ between males and females, XX for female and XY for male. All cells in our body have two sets of chromosomes, 23 from each parent.

Each chromosome pair have the same set of genes. Genes are sections of DNA that serve as units for hereditary information that contain sequences of nucleotides that encode the synthesis of a protein or RNA product with a specific function. A gene can have multiple variants called alleles, that occupy the same locus position on the homologous chromosome. A locus is a fixed place on the chromosome where a gene for a given trait occurs, e.g., the gene that decide the person's blood type (ABO-gene) have a fixed locus, in this case a specific place on chromosome 9, and has tree alleles A, B and O (Sjøberg, 2006, p 18).

Cells that have two sets of chromosomes, one from each parent, are diploid cells. Diploid organisms have two alleles at each genetic locus, one inherited from each parent. If the two alleles are identical the organism is homozygote at that locus, if the two alleles are unlike it is heterozygote. When an organism is heterozygote there is a dominant and a recessive allele. The dominant one is the allele that will always overrule the other recessive allele, but there are also instances where an organism can be heterozygote with two dominant alleles. Our blood type is such trait. Both A and B are dominant and will result in the blood type AB, while O is recessive.

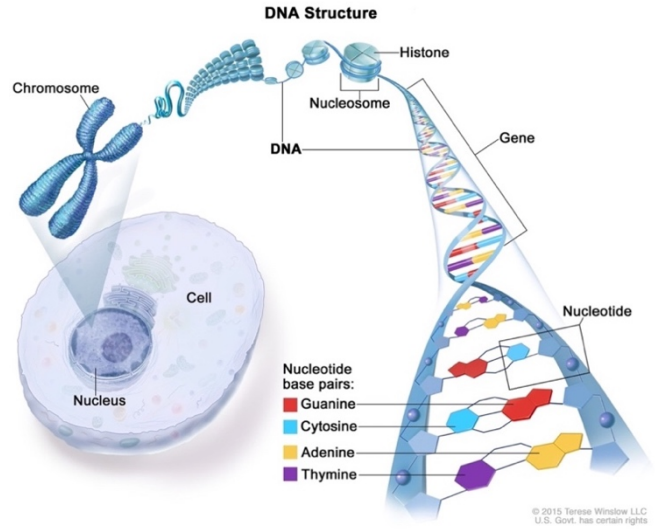


Figure 1: Structure of DNA. Winslow, Terese. (2015).

Retrieved April 4, 2022, from https://visualsonline.cancer.gov/details.cfm?imageid=10062

The basic building blocks of each DNA strand are the nucleotides. A nucleotide consists of three different chemical groups: a phosphate group, a sugar molecule (deoxyribose) and a base. A 3'→5'phosphodiester bond joins the 3'-hydroxyl group of deoxyribose of one nucleotide to the 5'-hydroxyl group of the deoxyribose of an adjacent nucleotide through a phosphoryl group (figure 2), this will result in a long unbranched chain with polarity. The DNA molecule exists as two strands that are coiled around a common axis making a long double stranded helix. The chains are paired in an antiparallel manner meaning the 5'-end of one strand is paired with the 3'-end of the other strand. (Harvey, 2011, p 395-398).

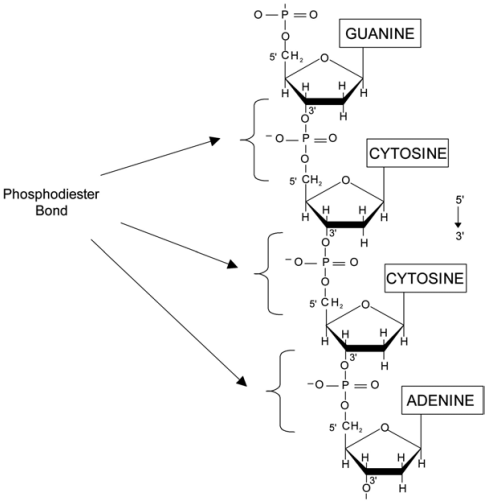


Figure 2: schematic presentation of phosphodiesterbond between two nucleotides (adapted from LEHNINGER 1985).

Retrieved April 1,2022 from "https://www.researchgate.net/figure/Schematic-representation-of-phosphodiester-bond-between-two-nucleotides-adapted-from_fig1_31968381

The base can be one of the so-called purines; adenine (A) or guanine (G), or one of the pyrimidines; cytosine (C) or thymine (T). The bases of one strand are paired with the bases of the second strand. Adenine is always paired with thymine and a cytosine is always paired with a guanine by hydrogen bonds.

The main function of DNA is to encode a functional protein, but only a small fraction (about 1 %) of the DNA is required for this purpose (MedlinePlus, 2021). Most of the DNA in a gene consists of non-coding sequences, also known as “junk DNA”. This non-coding DNA serves functions such as control of gene activity, it contains sequences acting as regulatory elements determining when and where specific genes are turned on or off. The non-coding DNA also makes the basis for the DNA profiling principle, which targets specific fragments called short tandem repeats (STRs) or microsatellites in the non-coding region. STRs are highly polymorphic regions consisting of 1-6 base pairs and occur up to 50 times in different individuals (Fan 2007; Alberts, 2010, p 316-320).

DNA is also found in the mitochondria of the cell. The mitochondrial DNA (mtDNA) is circular and double stranded. Unlike the nuclear DNA, which is inherited from the mother and father, the mtDNA is inherited only from the mother (Merheb, 2019). The nuclear DNA occurs in two copies per cell, while the mtDNA has as many as 100,000 copies per cell (Solomon, 2011, p 340).

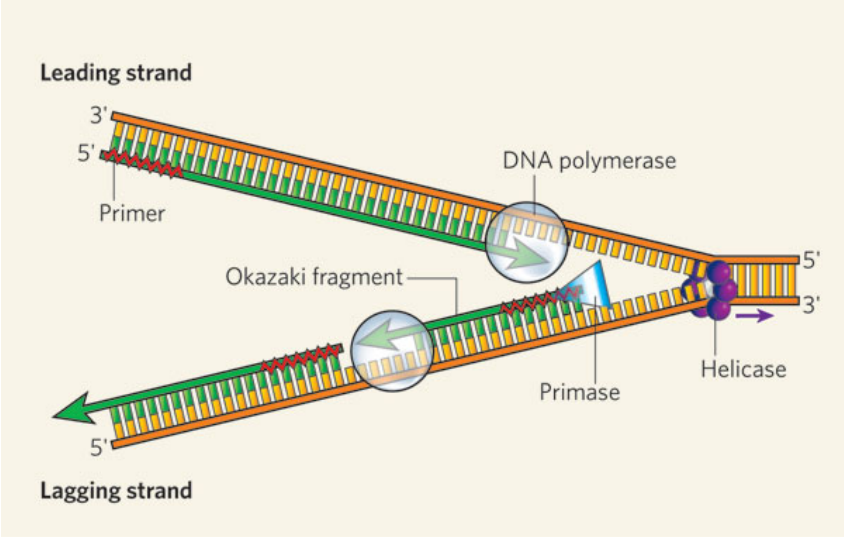
Over time, unless frozen, desiccated or preserved the DNA in cells will degrade over time. At a point PCR analysis of nuclear DNA will no longer be successful for DNA profiling, in these cases forensic scientists turns to mtDNA analysis. Sampling of mtDNA from degraded human remains e.g., decomposed, or skeletonized old remains where nuclear DNA is degraded has been shown to provide a useful DNA profile in many cases. Therefore, analysis of mtDNA has become the method of choice for anthropologists working with ancient skeletal material (Foran, 2006).

It appears that over a long period of post mortem interval mtDNA degrades at a slower rate than the nuclear DNA (Higgins 2015). The presence of a higher number of copies of mtDNA compared to nuclear DNA explains why mtDNA can be used for analysis in cases where nuclear DNA is too degraded. In addition, the location of the mtDNA inside of the inside the mitochondrial organelles also has a protective function against the DNA degradation (Goodwin, 2016, p 143-144).

DNA synthesis

The genetic information in the DNA molecule is copied and transmitted to daughter cells through DNA replication. The result is two double stranded DNA molecules that consist of one parent strand and one daughter strand. For the strands to be replicated they must be separated. The origin of DNA replication is at a specific nucleotide sequence called *ori* (origin of replication). In eukaryotic DNA there are multiple ori providing rapid replication. As the two strands are separated a replication bubble is made, and the DNA synthesis will occur as two replication forks move away from the ori bidirectionally. It is the enzyme *DNA helicase* (figure 3) that moves into the double stranded DNA molecule and forces the two strands apart.

The DNA polymerase enzymes are responsible for copying of the template strands, and they can only copy the parental strand in the 3'→5' direction and synthesize the new DNA strand only in the antiparallel 5'→3'- direction. DNA polymerase (Pol III) cannot initiate synthesis of a new strand, it can just elongate an already existing fragment. Therefore, they require an RNA-primer that consist of RNA base pairs. The RNA-primer is synthesized by an RNA polymerase enzyme called *primase* (figure 3). When the RNA-primer is added to the template the DNA polymerase can start the synthesis and elongation of the new DNA strand by adding nucleotides at the 3'-end decided by the template strand. The DNA polymerase elongates the chain by adding nucleotides and moving along the template strand. The RNA-primer consist of ribonucleotides instead of deoxyribonucleotides which later will be removed and switched to deoxyribonucleotides also by a DNA polymerase (Pol I).



Figur 3: DNA replication of the leading and lagging strand (Nature)

Retrieved May 11, 2022 from https://www.nature.com/scitable/content/dna-replication-of-the-leading-and-lagging-14668888/

The DNA strands are antiparallel meaning they run in opposite directions. One strand, the leading strand, undergoes continuous replication away from the replication fork. The other strand, the lagging strand, will have a discontinuous replication. The primers bind to the lagging strand and the DNA polymerase copy the strand until meeting another primer. The discontinuous replication makes small fragments of DNA called Okazaki fragments (figure 3). The gaps between the fragments are later joined by the enzyme DNA ligase to produce a continuous strand (Harvey, 2011, p 396-405).

Distribution of DNA within teeth

In literature, unrestored molar teeth are described as the best teeth for harvesting DNA material due to their large pulp chambers. However, usable quantities of DNA have been extracted from all types of teeth, even those with restorations (Adams, 2014, p 108-109). Extraction of DNA from human remains is a complicated task, and it depends on the state of the remains. The yield of DNA from the various dental tissues varies both in quality and quantity (Mansour, 2018).

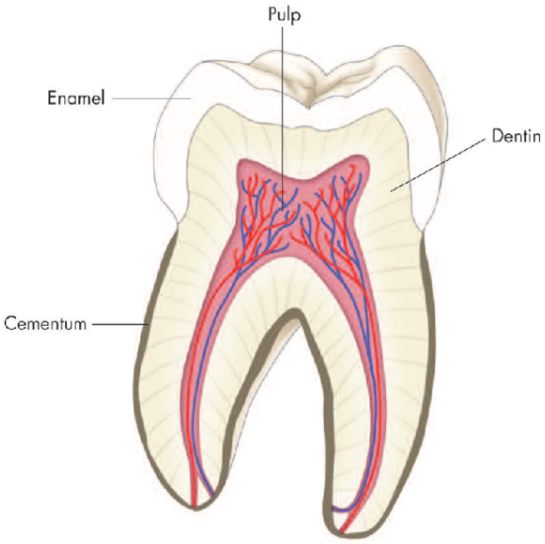


Figure 4: Major tissues of the tooth

Retrieved May 10, 2022, from https://www.semanticscholar.org/paper/Normal-Aging-of-Teeth-Figure-1-%3A-Major-Tissues-of-2/fb0011debb1445fbfc84656f93189860be4afcb4

Tooth substance consist of enamel, dentin, cementum, and pulp tissues. The enamel is the hardest tissue of the body consisting of 98% mineral (Higgins, 2013). The enamel is acellular meaning it will not contain any cells with DNA. Its strong composition protects the pulp and dentin cells by working as a protection against external conditions like heat, UV-light, humidity, and pathogens (Higgins, 2013; Malaver, 2003).

The dentin is composed of 65% mineral and does generally not contain nucleated cells (Higgins, 2013). It is hollowed out by the dentinal tubules which contain odontoblastic processes, nerves, and dendritic cells. (Malaver, 2003). The odontoblastic processes project from the odontoblasts, located in a layer right under the dentin, entering the dentin and follow the dentin tubules until the enamel-dentin junction. The odontoblasts are responsible for dentinogenesis and can also become trapped in the dentin in the formation of tertiary dentin. (Mansour, 2018). The odontoblastic processes are rich in mitochondria and are well protected due to their location inside the tubules. The dentin is therefore a good source of mtDNA, but a poor source for nuclear DNA (Higgins, 2013).

The dental pulp is a highly cellular tissue and will therefore be the main source of nuclear DNA in the tooth (Higgins, 2013). Some of the cells are the odontoblasts, dendritic cells, fibroblasts, undifferentiated mesenchymal cells, endothelial cells, macrophages, and lymphocytes. All of these are potent sources of DNA (Girish, 2010). Nerves, lymphatic- and blood vessels are also present in the pulp chamber, as they enter the tooth via apex and accessory canals (Higgins, 2013). The pulp is well protected by the hard tissues, but it is still affected by putrefaction after death and will eventually become totally decomposed (Malaver, 2003). Due to the pulp's high number of cells, it is subjected to dramatic degradation in a relatively short post-mortem interval. A study by Vavpotič states that the density of odontoblasts is inversely proportional with the time since death. The results from the study showed that "the relative density of odontoblasts after death drops by 1,025 percentage points per hours on average at room temperature and 1,015 percentage points per house in refrigerated conditions" (Vavpotič, 2009, p 125).

Cementum is the tissue that covers the root of the tooth, and is an avascular mineralized tissue composed of 40-50% inorganic mineral. Cementum is constituted by cellular and acellular cementum (Higgins, 2013). Cellular type provides a source for nuclear DNA due to the presence of cementocytes. As a rule of thumb, one can say that acellular cementum predominates in the coronal half of the root, while cellular cementum mainly covers the apical half of the root. The cementum has similar structure as bones, but does not contain innervation, blood vessels or marrow spaces (Mansour, 2018).

The amount of DNA yield from different tissues depends on environmental factors and the post-mortem interval (time since death), but the rate of degradation is impossible to predict because there are so many influencing factors. Mitochondrial DNA degrades at a slower rate than nuclear DNA (Higgins, 2015). DNA can be degraded by several factors including time, temperature, humidity, light, chemicals, and biological contamination (Hinchliffe, 2011). Therefore, the teeth with the greatest quantity of dental pulp-, cementum- and dentin tissue should be selected for post mortem DNA fingerprint analyses. This is typically an intact, pathology free molar tooth. Furthermore, molar teeth are better mechanically protected against the environment by the tongue, buccinator muscle and facial soft tissues compared with front teeth (Adams, 2014, p 107). The molars also have the largest root surface area. The use of molar teeth in identification is also recommended by INTERPOL in the Disaster victim identification guide (INTERPOL, 2018, p 12). Teeth that are going to be used in forensic identification should be placed in a sterilized container until the process of DNA analysis to avoid contamination.

Apart from obtaining a DNA profile of an individual, teeth can provide with another significant information used in forensic identification (Chowdhury, 2018). During tooth development, the amelogenin gene (AMEL) is expressed and a group of protein isoforms called amelogenins are synthetized. Amelogenins are believed to have an important role in the formation of tooth enamel. AMEL has two homologous genes; AMELX, which is located on the X chromosome, and AMELY which is located on the Y chromosome. After amplification of the AMEL gene, if only the shorter AMELX fragment is observed this will indicate an individual with only X chromosomes, usually predicting a female phenotype. If fragments of both lengths of AMELX and AMELY is observed it will indicate an individual with both X and Y chromosomes, usually predicting a male phenotype. The length variations of AMELX and AMELY make the basis of sex determination using amelogenin in forensic analysis (Dutta, 2017, Krishan, 2015). Thus, DNA from teeth can be used to determiuned the gender of the person the tooth belongs to.

PCR

In the next section of the paper, we will explain the principle of PCR and DNA profiling to get a better understanding of the practical experiment being presented later.

PCR (polymerase chain reaction) is a method to amplify a desired DNA sequence from a biological material. The PCR technique was developed in 1985 by Kary B. Mullis who later received the Nobel Peace Prize in chemistry in 1993 for his discovery. Today both DNA and RNA can be used in this technique (Kubista 2006).

In a standard PCR analysis, a template DNA is mixed with deoxynucleotides, a DNA polymerase enzyme and primers. PCR is performed by temperature cyclin and is carried out in 3 steps: (Sjøberg, 2006, p 176).

1. Denaturation

The samples are heated up to about 93-95°C. At these high temperatures the DNA template will be denatured, the hydrogen groups between the two strands breaks and the double stranded DNA will unwind. This will leave two single strands of DNA so they can be replicated individually (Senn, 2010, p 108; Porta, 2012;).

2. Annealing

In the next step the temperature is decreased. The annealing temperature can be between 48-72°C and is determined by calculating the melting temperature (Tm) of the selected primers, which is based on the composition and length of the primers. This is important to keep in mind when designing primers as both primers should ideally have similar Tm. The selected temperature will allow primers to attach to the specific locations of the template DNA (Sjøberg, 2006, p 177).

3. Elongation

In the last step the temperature is elevated to 72-75 °C to make the right environment for the heat-stable Taq polymerase, a DNA polymerase enzyme, which adds nucleotides by following the template strand. The enzyme will continue from one primer to the ending of the other primer. After this step the first cycle of PCR is completed (Harvey, 2011, p 482).

When the elongation step is finished the first PCR cycle is completed. The cycle is then repeated, doubling the previous amount of DNA, which continues with each subsequent cycle. A limitation of the PCR technique is that it is extremely sensitive, and even a small amount of contaminant DNA may become amplified. Therefore, it is very important to take precautions and work in a sterile environment to avoid technical errors (Solomon, 2011, p 331).

DNA profiling

The use of DNA fingerprinting in forensic science was brought to light by prof. Alec Jeffreys in the early 80s after his discovery of hypervariable loci using restriction fragment length polymorphism (RFLP). RFLP is useful for analysis of VNTRs (Variable Number of Tandem Repeats) which are unique patterns in DNA fragments making it possible to differentiate between individuals (Rasmussen, 2012).

After amplifying a DNA sample using the PCR-technique, it is cut into smaller pieces at specific nucleotide sequences (restriction sites) by restriction enzymes (restriction endonucleases) (Varsha, 2006). Because of the hypervariable polymorphisms of the non-coding regions, the length of the DNA fragments produced from enzymatic digestion will be unique for everyone (Jarcho, 2001).

According to size, the non-coding sequences can be divided into short tandem repeats (STRs) if the repeating unit is < 6 bp and variable number tandem repeats (VNTRs) if longer (Bakhtiari, 2021). In the early 1990s, PCR was used to type a lot of VNTRs regions, but over the years the technology moved towards the use of STRs due to their smaller size and a great level of stability.

Patterns of the repeated sequences are subject to stable inheritance (Sakari, 2015). An individual will essentially have two variants of inherited repeat sequence – one from each parent. This results in different combinations of paternal and maternal non-coding variations (Varsha, 2006).

The aim of the DNA-fingerprinting analyses is to confirm the presence of a set of specific DNA markers. Those DNA markers are standard, single-stranded sequences of DNA or RNA

that bind to the complementary STR-sequences in the DNA sample we want to compare. Thus, a unique pattern for an individual can be created based on how a series of markers binds to a DNA sample. This provides an opportunity to match biological samples obtained from a deceased individual against samples from AM evidence or a suspect (Sakari, 2015).

To compare DNA fingerprints, the DNA fragments must be separated. For this purpose, capillary electrophoresis (CE) is performed. Separation in CE is based on the electrophoretic migration differences of solutes under high voltage based on their size and charge (Ban, 2014). CE is typically carried out with capillaries of 50 μm in inner diameter, 150 to 350 μm in outer diameter and about 30 cm in length, made from high-purity fused silica. A capillary holds an aqueous buffer which acts as an electrolyte and makes it possible for the DNA fragments to be separated by their size (Ban, 2014). A sample is then introduced into the capillary. In an aqueous environment (buffer solution) the DNA molecule is negatively charged and will therefore move from the negative electrode towards the positive electrode after an electric field is applied. Smaller fragments will move faster than the large ones. Near the outlet end of the capillary, the separated fragments are detected, and the results digitalized and visualized as an electropherogram (Baker, 1995, p 19-37; Durney 2015).

An electropherogram is a chart that visualizes the separated DNA-fragments of various sizes as peaks with different migration times through the capillary (Baker, 1995, p 19-37). Thus, a unique DNA profile is generated. If enough STR markers are compared, the chance for two randomly selected people to have identical DNA may be one in a billion (Solomon, 2011 p 340). After European standard, a set of 17 different markers is used to establish genetic makeup of a person. According to the Norwegian Biotechnology Advisory Board, a complete DNA-profile can establish positive identification of an individual with almost 100% certainty. Identification of monozygotic twins is an exception from this general rule, as they will present with an identical genetic material (Bioteknologirådet, n.d).

Practical experiment

Introduction

To obtain a more profound understanding of the use of teeth for DNA fingerprint analysis in forensic sciences, we got the opportunity to perform a small laboratory test at the section of Forensic Genetics – Relationships and Identity, Oslo University Hospital. The test was performed over three days in March 2022.

Materials and methods

Sample collection

For the experiment, we extracted the upper left 3rd molar from one of the authors (AZZ) in the student clinic at the Faculty of Dentistry in Oslo. The tooth had a therapeutic indication for removal due to a buccal direction of eruption causing plaque accumulation and blisters of the gingiva of the cheek. The tooth had no dental pathology and was removed in toto by the other author (HHZ). The tooth was stored according to protocol in a cold and dry environment in a locked plastic container for approximately 4 days before it was taken to the laboratory. At the laboratory, a swab test from the buccal oral mucosa of AZZ was taken to compare her DNA profile with what we were to harvest from the tooth.

All the following steps were done following a standardized protocol developed by Oslo University Hospital. To prevent contamination all pre-PCR work, including the elimination of contamination of the tooth was performed in separate rooms dedicated to the specific task performed. Following the protocols appropriate protective wear also had to be worn by all personnel entering the room. Furthermore, all the equipment used was covered with plastic or were single use items and the workbenches were cleaned with sodium hypochlorite before and after the experiments were performed.

Elimination of contamination by sodium hypochlorite

Prior to extraction of DNA the tooth had to be cleaned. The tooth was carefully washed with 14% sodium hypochlorite, MilliQ-water (purified water) and 96% ethanol to remove contamination on the tooth surface. Thorough purification is an important step since insufficient removal of contamination can lead to false positives and error results. Sodium hypochlorite is used to remove exogenous DNA contamination from the surface of the tooth

by cleavage and breakage of the DNA-strands, leaving only the endogenous DNA from the internal dental tissues intact (Kemp, 2005).

Crushing and grinding of tooth

After the tooth had been washed it was placed in a cabinet to dry for at least 24 hours. The tooth had to be completely dry before proceeding to the next step. Then the tooth was crushed into smaller pieces by use of a manual bone crusher. Next, the smaller pieces were put into a grinder that pulverized the tooth.

A few drops of EDTA were used to gather the tooth powder from the grinder. A pea size of tooth powder was transferred into a test tube with a solution consisting of G2 buffer (lysis buffer which breaks up cell membrane structures to make DNA available), Proteinase K (removes contaminations from various proteins present in the solutions) 0,5M EDTA pH 8,0 (inhibits degradation of DNA) (El-Ashram, 2016). The solution and the powder were mixed by turning the test tube upside down a couple times. Then, the test tube and control sample were incubated at 56ºC and constant mixing for 24 hours.

DNA extraction

DNA extraction is performed to remove the DNA molecule from the biological source, in this case the tooth powder. After incubation the viscous supernatant was retransferred into a column to centrifuge so even more tooth powder were separated from the supernatant. The solution was placed in the EZ1 advanced machine by QIAGEN that performs nucleic acid purification using the DNA investigator kit-program for forensic and human identity samples. The EZ1 advanced uses magnetic bead technology as extraction technique.

DNA binds to the surface of the magnetic beads while other contaminants stay in the solution. This will result in purified DNA that can be used in amplification. Purified DNA performs well in downstream analyses such as PCR and STR analysis. The use of magnetic particle technology provides high-quality DNA, it works in a way where DNA is separated from lysates using a magnet (Biocompare, n.d). The instrument can run up to 6 or 14 samples at the same time.

PCR analysis

At the Institute of Forensic Medicine at Oslo University Hospital the PCR analysis is carried out using the PowerPlex ® Fusion 6C system by Promega. The system provides with a complete amplification kit for human identification and is used among others in paternity testing, DNA profiling as well as gender determination (Boavida, 2018).

The kit has a six-dye multiplex system allowing amplification and detection by fluorescence of the 23 autosomal loci (STR markers): CF1PO, FGA, TH01, vWA, D1SN656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, Penta D, Penta E, D22S1045, TPOX and SE33, as well as gender marker Amelogenin and STR-markers at Y chromosome: DYS391, DYS570 and DYS576 (Oslo University Hospital, 2022)

All samples were amplified using PowerPlex ® Fusion 6C system according to manufacturer's protocol. The PCR products can be stored in a dark freezer, storage in higher temperatures can lead to degradation or artefacts (Oslo University Hospital, 2022).

Capillary electrophoresis

The PCR products were centrifuged before starting the capillary electrophoresis. A plate with Hi-Di formamide (highly deionized foramide used to stabilize denatured DNA samples), ILS (ionic liquids in the electrolyte solution) and allelic ladder (mixture of alleles of known sizes and sequences from the PowerPlex ® Fusion 6C kit) was made, and different concentrations of PCR product were added (1-2μl). The new reagent was centrifuged to remove air bubbles and then heated up to 95°C for 3 minutes to denature the DNA. After the 3 minutes the plate was cooled down immediately. The samples were then run in the capillary electrophoresis instrument; Genetic Analyzer 3500cl, REFA by Applied Biosystems® (Oslo University Hospital, 2022).

Digitalization and comparison

In the laboratory the results from the DNA analysis of the tooth and the swab test of the oral mucosa were digitalized using the software GeneMapper® by Applied Biosystems. This software makes it possible to analyze the DNA profile that results from the capillary electrophoresis done by Genetic Analyzer 3500cl.

Results

The result from the practical experiment is showed in figure 5 and 6. The results are DNA profiles from AZZ´s extracted 3rd molar and buccal swab test. Figure 7 represents the DNA profile of a positive standardized control sample compared to AZZ's DNA profile collected from the extracted 3rd molar.

The peaks represent the presence of human DNA and different alleles. The alleles match when their peaks fall at the same position on the charts. Comparing figure 5 and 6 we can see that the peaks are matching in position. We can therefore conclude that the two profiles contain DNA-material from the same person, meaning that the positive identification could be established in a hypothetical identification process. This is of course no surprise, since we know that the two samples were taken from the same person, but the results may be similar to what can be seen when performing DNA fingerprinting in identification work.

In figure 7 we see two electropherograms where the peaks do not overlap in positions, meaning that the DNA profiles do not match, and a positive identification cannot be established. A positive control sample with a known DNA profile obtained using standardized protocol under the same laboratory conditions as the main experiment is always used in this kind of evaluations. The reason for this is to assure that the results are valid. If the control sample is contaminated with exogenous DNA all samples would have to be deemed inconclusive and the trial must be repeated. In our case, the difference between the DNA profiles from AZZ and the positive control works as a visual and pedagogical example on how two different individuals can be distinguished in a DNA analysis.

One of the loci analyzed is the amelogenin (AMEL) which is used for sex identification of the sample contributor. As seen in figure 7 the first-row sample (positive control sample) indicates a male showing X and Y versions of the allele, while the row below presents with only the X version, indicating a female (AZZ).

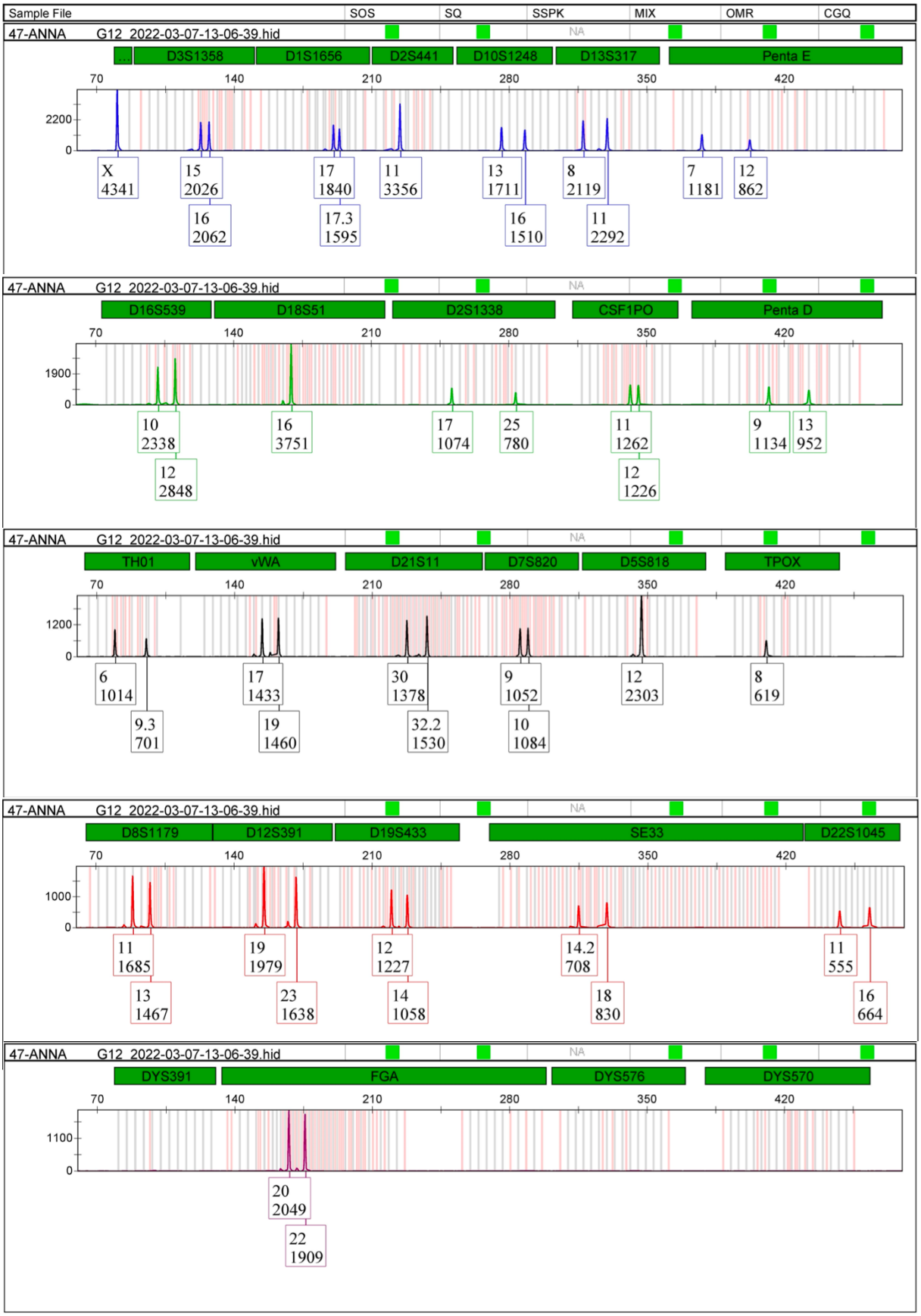


Figure 5: Electropherogram of DNA from buccal swab test recorded from AZZ

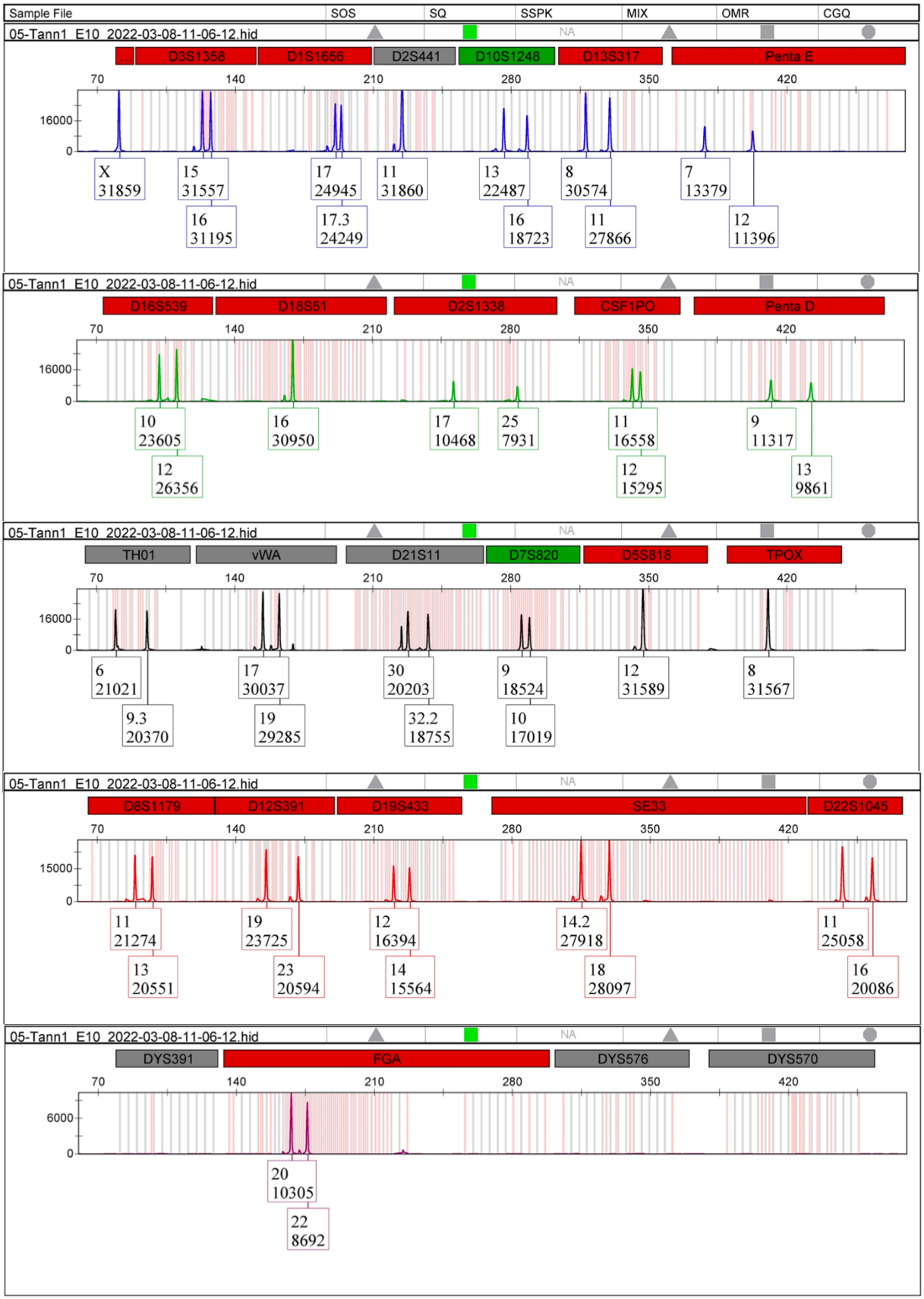


Figure 6: Electropherogram of DNA from AZZ's extracted tooth.

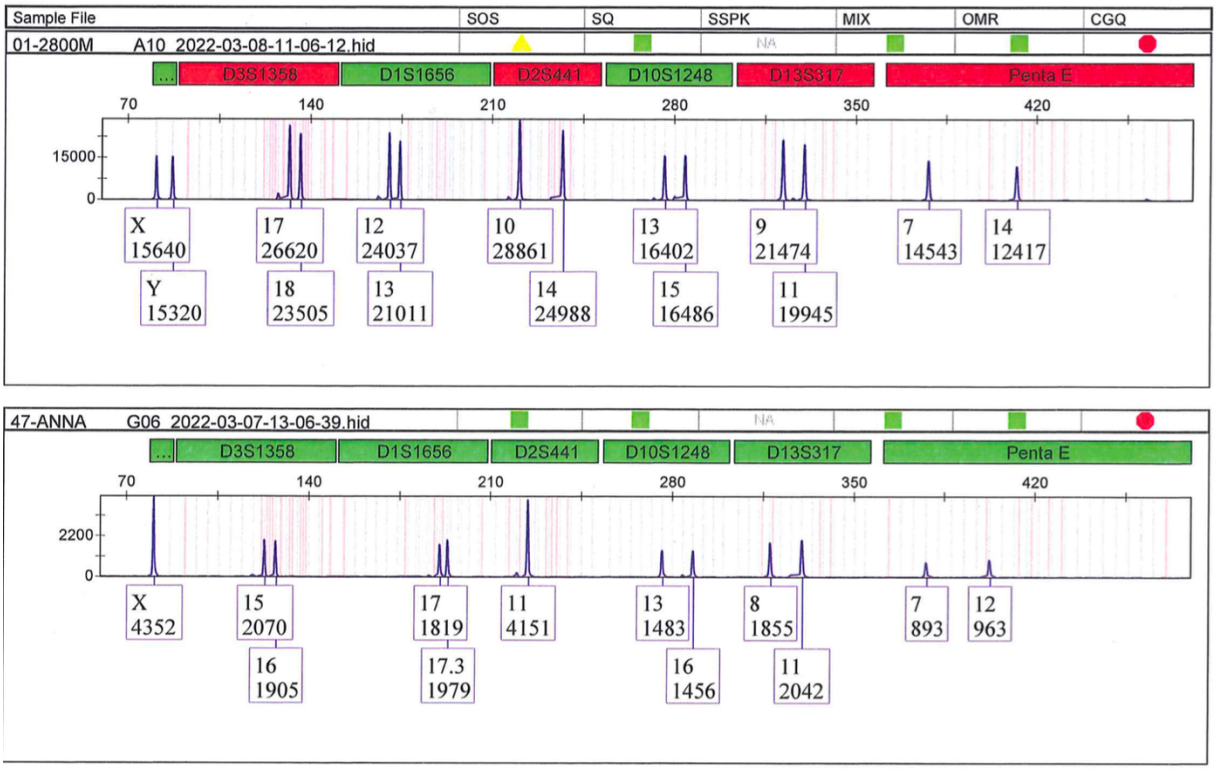


Figure 7: Comparison the electropherograms of DNA from positive control sample and AZZ.

Discussion

Data interpretation of the results of a DNA analysis is the most time consuming and difficult step. It requires expertise in the field and the results should be evaluated carefully. In STR analysis, the sequenced DNA fragments are labeled with fluorescent markers making them detectable during the electrophoresis process. After such recognition, the system correlates them to a standard of known fragments. The DNA profile consists of series of peaks that correlate to these fragments, sorted by size and dye color. The position of the peaks indicates which alleles are present. Pairs of peaks usually mean heterozygosity at that locus on the molecule, while single peaks indicate that the individual has only one variant at that locus and is therefore homozygous for that allele (Senn, 2010, p 111). Because each STR is highly polymorphic it will be slightly different for everyone (Adams, 2014, p 108). Another feature we see on the electropherogram results is the number under each peak. Those are computer-generated labels that tell us which particular allele each peak represents and how high the peak is relative to the baseline (Thompson, 2003).

When the sample contains a high quantity of DNA the peaks are easy to read. DNA can be degraded over time, and even faster in cases where the sample is placed in unfavorable

conditions. Degraded samples may present with too small quantities of DNA to reach the threshold to be used for analysis, making the heights of the peaks too low to be distinguished from background noise. In these cases, it will not be possible to generate a complete DNA profile.

In the PowerPlex ® Fusion 6C system by Promega that was used in this experiment, the sex of the contributor to the sample is identified by either a single peak or double peaks. A single peak showing "X" correlates to the profile of a female since females have a set of two X chromosomes. When double peaks are showing, one "X" and one "Y", the profile is of a male since males have one X chromosome and one Y chromosome.

Matching a profile from the deceased to the profile of a known reference AM sample will establish identity. Reference samples could be acquired from e.g., clothing, toothbrush, or hairbrush (Kolude, 2010). A forensic DNA typing can have three possible outcomes: match, mismatch, or an inconclusive result (Dash, 2020). Our laboratory experiment shows us that non-decomposed teeth without pathology, like Anna's extracted 3rd molar, have enough quantity of nuclear and mitochondrial DNA to receive a full worthy DNA profile giving a match of the DNA profile of the tooth and the buccal swab test (Higgins, 2015).

Summary

In forensic cases where human remains are decomposed, bones and teeth may be the only source of DNA available for analysis (Dutta, 2017). Teeth are more likely than bones to withstand different environments over long periods of time due to their morphological, anatomical, and histological structure, and their "protected" location in the alveolar bone (Krishan, 2015; Shah, 2019). Teeth will therefore experience less contamination and DNA degradation compared to bones (Mansour, 2018) and are a more reliable source of DNA for forensic identification. Since forensic odontologists are considered experts of the teeth, it is important that they have some knowledge about the possibilities of harvesting DNA from teeth. We hope that our master thesis has shed light on this topic and made it a bit easier to understand for our colleagues in forensic dentistry.

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