Methods of Preparing the Tooth for DNA Isolation

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Summary

In this paper, the importance of the tooth in identification an unknown person has been demonstrated.

The theoretical part shows the difference between genomic and mitochondrial DNA. That difference is the durability and properties which they carry and the value they have in identification. Microanatomy has also been described, possible locations of cells in the tooth and methods to collect material for DNA extraction.

The practical part shows the methods used in particular cases for DNA isolation. Horizontal section through the cervical root is used to collect material for DNA isolation. Genomic DNA , from the material, is extracted by organic phenol-chloroform methods. The results are shown on 1 % agarose gel and photographed by a Polaroid camera.

Key words: *DNA, tooth, identification, forensic dentistry, AMEL gene, DNA isolation, phenol-chloroform organic method, horizontal section.*

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Introduction

In the late 1980's, with development of technology that is faster, more sensitive and more specific, the analysis of DNA caused a revolution in the field of forensic science, including forensic dentistry, anthropology and archeology, making identification easier and more accurate. Until then only dental identification had been used, in forensic dentistry. However, to identify someone through dental identification predeath dental records we needed (1,2).

DNA is a molecule that builds genes, so the gene carries all genomic information that determine inherited characteristics. In the nucleus of all somatic cells in the body we find a double stranded structure of genomic DNA. Most of the cells inside the body contain organelles located in cytoplasm called mitochondria, and each mitochondria contains several mitochondrial DNA, mtDNA, molecules.

A large number of mitochondria inside the cell increases the chance that the mtDNA will stay preserved for a longer period. It is the only means of identification if there are a few generations in-between people whose DNA are being compared (3,4).

Besides DNA typing the discovery of AMEL gene made sex identification even easier (5,6). A new method by the name of polymerase chain reaction or PCR made using even very small amounts of DNA possible (7,8,9,10).

Microanatomy of the tooth is very important for better obtainment of the samples for DNA extraction, as are the methods used to obtain the sample.

Pulp chamber, dentine powder, dentine-cement powder, cement powder, or adherent bone, periodontal fibers can be used as samples for isolation. The dentine-cement powder proved to be the best sample for isolation of either genomic or mitochondrial DNA, because the DNA in it remains well structured the longest and there is rarely any contamination with non-human DNA (11,12,13,14).

Methods currently used for obtaining samples for DNA isolation are: crushing the entire tooth, conventional endodontic access, vertical split, and horizontal section through the cervical root.

The horizontal section through the cervical root seemed to be the best, as it permits rotary instrumentation of the inner dentine in root canals, and independent sampling of the cement, while conservation of the coronal morphology is very important for it can be used for morphological identification (14,15,16). The purpose of this paper is to show in theory and practice how genomic DNA is isolated from cells of dental pulp and dentine-cement powder.

Samples and methods

Samples for isolation of the genomic DNA were three intact teeth: upper first left premolar,-24, upper second left molar, -27, upper first left premolar, -24. The teeth were extracted for orthodontic purposes in the Department Oral Surgery of School of Dental Medicine in Zagreb. The teeth were treated with 10% natrium-chloride (NaCl). Visual examination showed the absence of any caries or fillings.

Sample number 1, upper premolar, weight 1360 mg (Figure 1); sample number 2, upper molar with unfinished growth of its roots (2050 mg) (Figure 2); number 3, upper premolar (1510 mg) (Figure 3).

Samples were cut horizontally by a diamond plate in a micromotor in the area of the enamel-dentine joint. From the area of the pulp chamber and root canal we performed extraction of the pulp, which was placed in Eppendorf tubes (the samples named as 1p, 2p, 3p). Roots were ground in a tissue grinder to a fine powder and placed in a test tube (the samples named as 1d, 2d, and 3d). Crowns of the teeth were saved.

In DNA isolation from the pulp and dentine-cement samples two organic methods (phenol-chloroform) were used: one for the pulp samples (17), and the other for dentine-cement powder samples (18).

Case presentations

A. Isolation of the DNA from the pulp samples of the tooth listed as tooth number 1

After the conservation of the pulp samples in Eppendorf tubes begin to isolate the DNA. First add to the specimen 1 ml of the sterile deionized water, mix and centrifuge on the maximum number of twists for 5 minutes, after which move the liquid and repeat the procedure two more times, altogether three. In the tube add 450 µl of extraction buffer (10 mM Tris-HCl, pH 8.0; 10 mM Na2EDTA: 100mM NaCl, 2% SDS) and mix thoroughly. Add 30 µl of Proteinase K (Gibco Brl, Life Technologies, Gaithersburg, USA) to the final concentration of 0.67 mg/ml. Mix carefully and incubate for 18 hours at 56°C. After 18 hours add 30 µl Proteinase K, and incubate for 3 more hours at 56°C. After the incubation centrifuge it at 13000x g; 15 minutes. Remove carefully the upper aqueous layer, supernatant, and transfer it into a new tube. In approximately 450 µl of supernatant add 450 µl phenol/ tris saturated (SIGMA Chemical, St. Louis, USA) and mix thoroughly turning the tube up and down. Leave at room temperature for 5 minutes and then centrifuge at 3000 x g; 10 minutes.

Carefully remove the supernatant and transfer it into a new tube and add 225 µl phenol and 225 µl mixture of chloroform (J.T. Baker INC., Philisburg, USA)/isoamyl alcohol (Kemika Zagreb, Hrvatska) in proportion 24:1. Mix it and centrifuge at 3000 x g; 10 minutes. Take the supernatant and transfer it into a new tube and add 450 µl of the pure chloroform, mix and centrifuge again at 3000 x g; 10 minutes. Take the supernatant, transfer into a new tube and begin precipitation by adding 2.5 volume parts of 100% ethanol (Pharmco Products INC., Brookfield, USA), or approximately 1.2 ml, and 9 µl NaCl. Mix thoroughly and leave for 2 hours at -20°C. Centrifuge at 3000 x g/20 minutes. Carefully remove alcohol, add 1ml 60% ethanol,

and centrifuge at 3000 x g/10 minutes. Remove alcohol, and the tube with the sediment leave to dry overnight. After drying dissolve the sediment in 100 µl sterile redeionized water. Extracted DNA is tested by putting on 1 % agarose gel in 1 X TBE buffer (89 mM tris base; 89 mM boric acid; 2 mM EDTA, pH 8.0).

B. Isolation of DNA from dentine-cement powder of the tooth labeled as sample number 2

When we obtain the dentine-cement powder by grinding with a tissue grinder we put it in a polypropylene tube, and start the process of isolation of DNA. Add into the tube 3 ml of the extraction buffer (10 mM Tris-HCl, pH 8.0; 10 mM Na_2EDTA: 100 mM NaCl, 2% SDS) and 100 ml 20 mg/ml proteinase K(Gibco Brl, Life Technologies, Gaithersburg, USA), mix thoroughly, and incubate overnight at 56°C. After the incubation add 3 ml of phenol/chloroform/isoamyl alcohol (25:24:1), mix thoroughly and centrifuge at 5000 x g; 2 minutes. Transfer the aqueous layer into a clean tube and repeat the procedure until the interface is clean. After the interface is clean add 3 ml n-butanol (SIGMA Chemical, St. Louis, USA), mix thoroughly and centrifuge at 5000 x g; 2 minutes. After centrifuging remove n-butanol, in this case the upper layer, as precisely as possible. Transfer lower liquid layer transfer into centricon-100 concentrators (Amnicon, USA). Centrifuge again at 1000 x g; 30 minutes, or longer, if necessary. Discard the filtrate and add 2 ml TE buffer and centrifuge at 1000 x g; 30 minutes. Discard the filtrate and repeat. After the procedure, turn the centricon filter upside down. Add TE buffer to the sample (approximately 100 (l), until the isolated DNA has been concentrated. Extracted DNA is tested by putting on 1% agarose gel in 1 X TBE buffer (89 mM tris base; 89 mM boric acid; 2 mM EDTA, pH 8.0).

Results

After the preparation of the gel we begin the setting of the extracted DNA on the gel. We put the marker in the first compartment. For analysis of the genomic DNA we used a λ/Hind III (Gibco Brl, Life Technologies, Gaithersburg, USA) marker, because we expected a product of the approximate length 23 000 bp (basic pairs). We added a loading buffer to each sample a color consisting of two components (brom-phenolum blue and xilen-cianolum). After we finished putting the samples into compartments we poured the rest of the TE buffer into the tub and connected to the voltage (10 V/cm of the gel). When the electrophoresis wss over we put the gel on a UV transluminator (TFX320.MC, VILBER LOURMAT), and took a photograph with a Polaroid camera (FOTODYNE, USA) (film 667).

MWM v-Molecular weight marker number v;
MWM XIII - Molecular weight marker number XIII;
λ/Hind III - length marker;
1p - the sample of the tooth(s pulp number1;
2p - the sample of the tooth(s pulp number 2;
3p - the sample of the tooth(s pulp number 3;
1d - the sample of the tooth(s dentine-cement powder number 1;
2d - the sample of the tooth(s dentine-cement powder number 2;
3d - the sample of the tooth(s dentine-cement powder number 3; (Figure 4).

Discussion

DNA analysis caused a great revolution in the field of forensic identification (5). Around 20% of the whole human DNA consist of a sequence of nucleid acids which are very likely to repeat, so arecalled satellite DNA. In the process of evolution satellite DNA change quickly, and they change also places in cromosomes. The human genome contains at least several predominant satellite DNA sequences. The mixture of those sequences are found at each centromere. The number of copies of a satellite DNA vary over a wide range so that remarkable comparisons can be identified that discriminate between two persons. This biological discovery has led to a strategy and technology sufficient to discriminate between siblings and between siblings and their parents (9).

Discovery of the AMEL gene made us realize how easy it is to discover the sex of a person. The sex of skeletal bones or teeth can be rapidly determined with enormous accuracy in a segment of human X or

Y chromosome-encoded AMEL gene. There are differences in the amelogenin proteins synthesized from either the X or Y chromosome. The fact that the X- or Y. specific AMEL genes are 106 and 112 base pairs, or bp, in length, respectively, provides a relatively direct procedure to discriminate between male and female AMEL gene. On a bar-code type display, a male DNA sample appears as two discrete bands of 106 and 112 bp, and a female DNA sample appears as a single band of 106 bp for the AMEL gene (5,19,20).

The mtDNA represents 0.5 % of the total DNA and is easily separable from the genomic DNA. MtDNA is 16569 nucleotide bp in length and is present in high-copy number in all cells and is more likely to survive for prolonged periods, compared to chromosomal, genomic DNA (21).

MtDNA is very useful in forensic identification, as it is inherited only from the maternal line, and is the best way to test relation if there are several generations between ancestor and living descendant. Human DNA usually encodes 100 000 genes and mtDNA encodes only 13 different genes (22,23).

MtDNA analyses have extended the range of analysis from a few thousand years ago to more than 13 million years ago. MtDNA has been used to identify 7000-year-old brain tissue, 500-year-old bone tissue as well as the bones from the grave of the Romanov family killed in 1918. In the identification of Romanovs DNA used for comparison was taken from relatives of the Czar and Czarina which were connected with them only through a maternal line, and the bodies of the children were identified after identification of the one of Czarina. To make a successful identification we need a sample as small as 20 to 70 pikograms (5,24,25,26).

We must say that even this kind of identification has its flaws. First is the amount of time needed, then availability of the samples needed for comparison and so on. But, when all other methods fail, DNA analysis is always here as a very secure method of identification, as demonstrated in the identification process of the victims of the freedom war in Croatia 1991-1995 (27,28,29,30,31).

It is reasonable to expect new advancement in DNA technology, which would reduce time and costs needed in the fast search for identity of unidentified bodies.