

DETERMINATION OF SEX IN DENTAL PULP USING PCR

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INTRODUCTION

Dental pulp has proven to be a very good source of biological evidence for forensic casework. Its location in a especially protected cavity makes dental pulp very valuable. The diagnosis of the sex has been elected like parameter of study by being a binary variable of easy confirmation and included the only variable that could be contrasted in samples of human bones remains. This simple parameter allows to study the behavior of the DNA obtained of dental pulp subjected to different external agents and as well as the antiquity.

MATERIALS AND METHODS

532 pieces has been analyzed to teeth (the half of the masculine sex and the half of the feminine).

The extraction of DNA of the pulps was carried out for the method of the phenol/chloroform (Maniatis 1989). The amplifications were performed in a final volume of 25 μ l in a Perkin-Elmer thermocycler. Each reaction contained 20-500 ng of DNA relying of the characteristics of the samples, 1 U taq polymerase (Boehringer), 10 mM tris-HCl pH 8.3, 50 mM KCl, 1.5 mM Mg Cl₂, 200 μ M of each dNTP and 0.4 μ M of each primer X₁₄₅ and X₁₄₆ for the amplification of the fragment of the X chromosome and 0.1 μ M of each primer Y₁₋₃ and Y₁₋₄ for the amplification of Y-specific fragment. The cycles temperatures were as follows: 95 °C (1 min), 54 °C (1min), 72 °C (1 min), 28 cycles with an increment in the phase of extension of 6 seconds. Prior to the first cycle the DNA was denatured at 95 °C for 10 min and after the last cycle an

additional extension at 72 °C for 10 min was performed. This protocol was based in Pfitzinger et al. (1993) but carrying out the amplification for separating of the fragment of the X chromosome and the Y chromosome.

The fragments was visualized on submarines agarose gels with tris/acetic/edta (pH 8.0) 1x as buffer.

RESULTS AND DISCUSSION

A) Results in relationship to the state of the dental piece:

Number of studied pieces:198, conserved at room temperature, maximal antiquity one week.

The healthful pieces and with cavities, but without pulp damage they allow to get excellent results (100% of success), however the pieces with cavities and pulp damage they offer results but less satisfactory (94,73% of success).

B) Results in relationship to the class of piece in function of the concentration of obtained DNA:

Number of studied pieces: 160, conserved at room temperature, maximal antiquity one week.

A direct relationship between the volume of pulp is observed and the concentration of DNA extracted in each class of piece, returning the maximal efficiency at first molar.

C) Results in pieces subjected to incineration :

Number of studied pieces : 80, all were subjected to 8 levels of temperatures (100-800 °C) during 5 min.

In pieces subjected 100-200 °C during 5 min, the results have been satisfactory (100% of success).The pieces subjected to 300°C during 5 min. they offer difficulties in approximately the 50% of the cases (56% of success). To 400 °C during 5 min. the pieces could rarely have been studied (12,5 % of success). It through 500 °C has not been possible to us study the DNA of dental pulp.

D) Results in connection with the antiquity:

- pieces with an antiquity 0-2 years and subjected to different

conditions of conservation:

Number of studied pieces 378.

For an antiquity until 2 years, the frozen (-20 °C) is the method that offers the best result (100% of success), although the room temperature and the interment doesn't disable the study of the DNA. (98,98% of success and 98,33% respectively). The conservation in saline serum seems to affect the posterior study of DNA of more notable form (91,66% of success).

- pieces with an antiquity 2-20 years:

Number of studied pieces 68.

For their study we formed a group in intervals of years: from 2 to 5, of 5 to 10, of 10 to 15 and of 15 to 20; the results were as follows: 90,0%, 100%, 85,71% and 92,85% of success.

The results are not very uniform, probably due the conditions of coservation; this condition could not have been standarized, being in many cases unknown for us. All the pieces treated with oxygenous water must be discarded, since the DNA is destroyed completely.

We believe that in this type of samples should take special caution: increase the quantity of sample (2 pulps); use more efficient methods of extraction (e.g.: Centricon Perkin-Elmer); an accurately control of the method.

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