

## GENETIC IDENTIFICATION FROM DENTAL PULP BY USING DNA AMPLIFICATION (PCR)

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### INTRODUCTION

The particular dental envelope resistance to environmental factors and putrefactive phenomena allows to obtain idoneous DNA for amplification by PCR and to use these human remains for forensic purposes so, the dental pulp permits to acquire genetic profiles and to compare these with familiar patterns. In forensic science is also important to determine how environmental factors can effect the human DNA. This kind of study is not very represented in literature. Infact the recent introduction of PCR method allows to carry out these analysis using a small amount of substance and exceed the limitations of Southern Blot. To evaluate the possibility offered by dental pulp and the applicability of different genetic markers, the authors have typified DNA obtained from teeth subjected to various environmental conditions and they have studied three different markers: the DQalpha locus, a VNTR system (D1S80 locus) and a STR system (HumTH01).

### MATERIAL AND METHODS

#### Samples

The teeth were obtained from extraction performed on patients of the Dental Clinic of our University. The specimens were whether healthy or decayed, extracted for therapeutic (orthodontic) or pathologic (periodontology) reasons, from different teeth of patients variously aged. For comparison reasons some peripheral blood samples were prelevated.

#### DNA Extraction

DNA extraction was carried out using two different protocols: modified Chelex according to Walsh (1991) and the classical method of phenol/chloroform after an incubation with proteinase K (20 mg/ml).

#### Analysed system

DQalpha Locus: HLA DQalpha genotyping was performed with Amplitype HLA-DQalpha Forensic DNA amplification and typing kit. PCR was carried out at 94°C - 65°C and 72 °C for 30 cycles and the amplification products were identified by the dot blot technique according to Perkin Elmer protocol.

D1S80 Locus: The samples were amplified by using D1S80 Forensic Set by Perkin Elmer. PCR was carried out at 94°C - 65°C and 72°C for 30 cycles and the amplification products have been separated by agarose gel electrophoresis followed by staining with ethidium bromide

HumTH01 System: The amplification was carried out according to Edwards et al. (1992) with some modifications. The amplification fragments were separated by electrophoresis using poly-

crilamide gel (T 8% ; C 5%) followed by silver staining.

#### Condition of exposure

We exposed teeth to standard maintenance or aggressive conditions. The first group of conditions were: room temperature, refrigerator (4-8 °C), freezer (-20°C) whether immediately after extraction or within a period of conservation (less than three months) both under various humidity conditions (60-80-100%) and dry environment. The aggressive conditions were both chemical (Hydrogen Peroxide, Hydrochloric acid 0,01 M, Sodium Hydroxide solution 0,01 M, Sodium-Chloride and water) and physical (180°C, 250 °C 800 °C); the specimens were exposed for a short period (30 minutes) only in one half of sectionated teeth (the other half of each tooth was under standard maintenance conditions)

#### RESULTS AND DISCUSSION

Good results with Chelex extraction protocol were obtained with

DQalpha and TH01 locus studies, instead smear results followed the application of Chelex protocol with DLS80, but for this locus study good results were obtained with phenol-chloroform extraction protocol. DQalpha gave for all conditions good results with exception of the entire set of blue spot indicating the detection of DNA (but degraded and untypified) in the specimen exposed directly at 800° C for 30 minutes. The test executed for comparative reasons on an half of the tooth or on blood indicated always the precision of the technique. TH01 was carried out in the most interesting aggressive conditions giving similar results to DQalpha. DLS80 was supplying favourable answers only in standard maintenance conditions, and in exposition at 180° C too, but only with phenol-chloroform protocol; no results were obtained with the specimen submitted to 800° C.

Significative differences were found in all situations where a too small amount of substance (usefull and no atrophic matter) was disponible depending both on age of the patients (from 15 to 70 years) and on type of tooth (from inciseve to molars), and on integrity of pulp material (from healthy to seriously decayed and infected teeth).

#### CONCLUSIONS

The positive results obtained in this study encourage us to further application on different loci and in experimental aggressive conditions to simulate true cases of seriously damaged human remains analysis.

#### REFERENCES

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- 2) Walsh PS, Metzger AD, Higuchi R (1991) Chelex 100 as a medium for simple extrtaction of DNA for PCR-Based typing from forensic material. *BioTechniques* 10:506-513

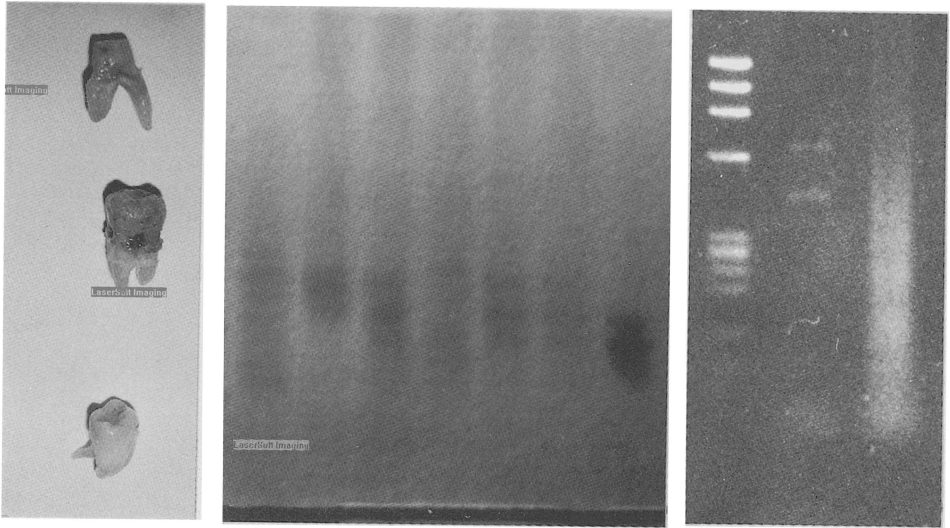


Fig. 1 : THE TEETH USED FOR THE EXTRACTIONS

Fig. 2 : STUDY OF HumTH01

Fig. 3 : STUDY OF THE D1S80 LOCUS (CHELEX EXTRACTION)

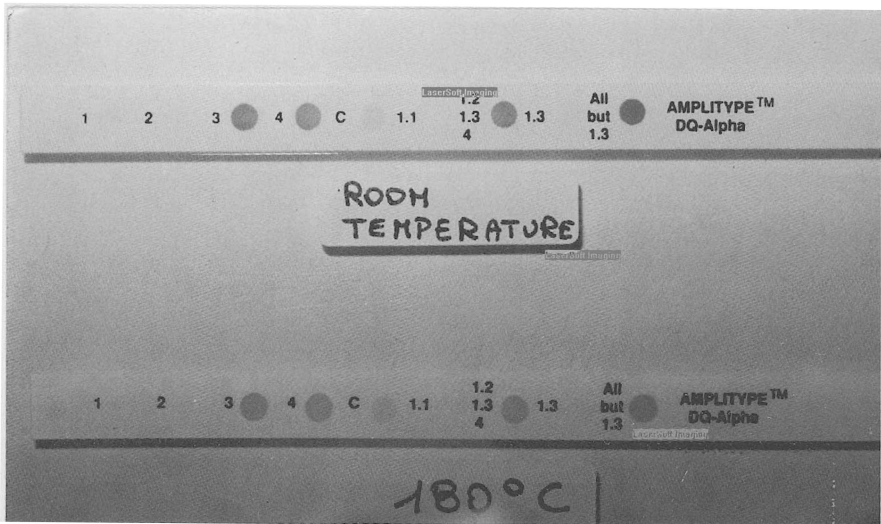


Fig. 4 : STUDY OF THE DQ $\alpha$  LOCUS