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Typing of teeth with two different amplification systems

N. Cerri[a,*], U. Ricci[b], A. Verzeletti[a], B. Falconi[a], F. De Ferrari[a]

[a] *Institute of Legal Medicine, University of Brescia, Piazazale Spedali Civili 1, 25123, Brescia, Italy*
[b] *Genetics and Molecular Medicine Unit, University of Florence, A.O. "A. Meyer", Florence, Italy*

Abstract. Two different automated procedures (capillary electrophoresis and infrared automated sequencer) for STR analysis on DNA obtained from highly degraded teeth were compared. Even with these two automatic protocols, negative or not much informative results were obtained. Our results confirm that in extreme conditions, it is necessary to perform more sensitive analysis (i.e. SNPs, mt-DNA) to obtain informative genetic profiles. © 2003 Elsevier B.V. All rights reserved.

Keywords: Teeth; STRs; Infrared and capillary electrophoresis analysis

1. Introduction

Many studies showed that the particular dental resistance to environmental factors and to putrefactive phenomena allows to obtain suitable DNA from dental pulp and to achieve a genetic profile for identification [1–3].

In the past, dot-blot analysis (Dqalpha and Polymarker), VNTR or STR with manual procedures showed differences in all situations where a too small amount of substance was available, depending both on the subject's age and on the type of tooth. When dental pulp was not integer (decayed or infected tooth), in carbonised specimens or in the case of teeth exposed directly to high temperature or aggressive conditions, no results were obtained [4].

In this study, DNA extracts, previously obtained from different kinds of teeth and evaluated by dot-blot analysis, were reanalysed with two different automated procedures: capillary electrophoresis in an ABI-Prism 310 instrument (Applied Biosystems) and an Infrared automated DNA sequencer LI-COR 4200 (LI-COR, Nebraska, USA).

* Corresponding author. Tel.: +39-30-3995480; fax: +39-30-3995839.
E-mail address: deferrar@med.unibs.it (N. Cerri).

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2. Materials and methods

Human teeth, extracted for orthodontic or periodontology reasons, were obtained from oral surgeons' patients of various age. We exposed teeth to standard and aggressive conditions (freezer, various humidity conditions, dry environment, chemical substance, carbonisation).

DNA extraction was carried out using two different protocols: Chelex method and phenol/chloroform method after incubation with proteinase K (20 mg/ml).

DNA was amplified and analysed by two different automatic procedures: 1. AmpFlSTR® Profiler Plus™, SGM Plus™ and Green I from Applied Biosystems kit, according to manufacturer's procedures, analysed by capillary electrophoresis in an ABI-Prism 310

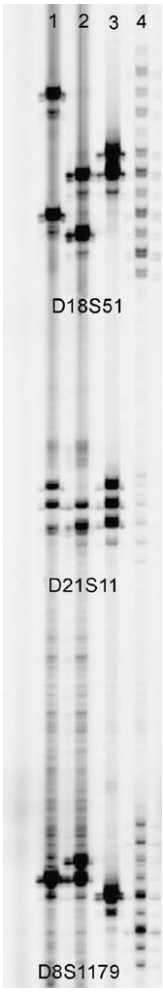

Fig. 1. Results obtained with MU3 system: 1—sample A; 2—sample B; 3—K562 cell line; 4—allelic ladder.

instrument (Applied Biosystems). 2. Amplification of four independent PCR multiplex systems and analysis with an Infrared automated DNA sequencer LI-COR 4200 (LI-COR).

Nonquantified extract (1 μl) was employed for the analysis, according to the previously described protocol [5,6]. For LI-COR analysis, we used the QIAGEN® Multiplex PCR kit to amplify the whole STRs of CODIS system. The amplifications were performed in a total volume of 12.5 μl, using 5.8 μl of Master Mix containing buffer, dNTPs, magnesium, HotStarTaq DNA polymerase and 5.8 μl of primer mix as previously described [5]. DNA (0.9 μl) was added before PCR. When samples gave negative results, 0.9 μl of a 1:10 dilution was used for PCR. The following amplification conditions were used: 95 °C for 15 min, then 94 °C for 30 s, 60 °C for 1 min and 30 s, 72 °C for 1 min and 30 s, for 32 cycles, with final extension at 72 °C for 10 min. Stop solution (6 μl) containing 95% formamide, 10 mM EDTA and 0.1% blue bromophenol were added at the end of the amplification cycles. Gel electrophoresis was performed in a LI-COR® model 4200 monolaser automated fluorescent DNA sequencer as previously described [5]. The result of the run appeared in an autoradiogram-like image as shown in Fig. 1.

3. Results and discussion

Our results do not seem to support significant differences between the two amplification systems. The minor variation in typing seems to occur because of a random fluctuation (e.g. pipetting). Good results with both methods were obtained in the samples that showed a positivity even by using a dot-blot analysis; no significative differences were found in all situations where a too small amount of substance was available nor in samples obtained from various age patients (presence or not of atrophic matter), from different type of teeth or with regard to the integrity of dental pulp (healthy or infected).

Even with these two automatic protocols, negative or no informative results were obtained when teeth were submitted to extreme conditions (e.g. incinerated tooth at 800 C°), while incomplete profiles were obtained in teeth exposed to 250 °C. Our results confirm that in extreme conditions, it is necessary to perform more sensitive analysis (i.e. SNPs, mt-DNA) to obtain informative genetic profiles.

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