PCR-DNA typing from beard samples

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Introduction

The analysis of DNA polymorphisms by means of PCR (Erlich H.A., 1989; Innis M.A. et al., 1990) has drastically increased the possibility of using some evidence that was not suitable until a short while ago.

Moreover, the introduction of STRs (Edwards A. et al.; 1991), HLA-DQα (Saiki R.K. et al., 1986) and Polymarker system (Amplitype^R PM user guide, 1994) kits in forensic applications has greatly improved the discriminating power of these tecniques also in the case of a small amount of target DNA.

In this study we tried to extract, amplify and type DNA from the material remained after shaving, on blades and inside the electric razors. We think this kind of samples could be considered an interesting specimen about identification aims in forensic biology.

Materials and methods

We used both electric razors and blades specimens, sampling, at the end of a single shaving, the material remained inside the electric razor or between the blades of 10 individuals. Besides we took their blood as control.

Samples of material obtained from electric razors (Braun and Philips) were examined with the light microscope and the Scanning Electon Microscopy (Cambridge 110) to seek for the presence of cells, expecially nucleated ones. We collected the material with bi-adhesive tape stuck on a slide for the observation with the light microscope and on stub for the SEM. The former samples were stained with toluidine blue at 60°C, while the latter were gold-coated. As to the samples obtained from blades, to avoid possible inhibition by chemicals in shave soaps, this material underwent successive washings and centrifugations to pellet the skin evidence. The hair fractions were separated from the cells at low centrifugal force (2-3 g) to eliminate the hair constituents that could interfere with PCR (Yoshii T. Et al., 1993). The pellets were resuspended adding 300 µl of lysis buffer (Tris 0.01 M, pH 8.0, EDTA 0.01 M, NaCl 0.1 M, DTT 0.06g/ml, SDS 2%).

After a 3-hour incubation at 56°C, the evidence was digested with 10 µl of proteinase K (20 mg/ml) at 37°C overnight. According to our previous experience on the same kind of evidence the extraction was performed following the "salting-out" protocol (Miller S. et al., 1988), that proved to be more efficient than phenol-chloroform (Sambrook J. et al., 1989) and Chelex^R ones (Walsh P.S. et al., 1991). DNA extracts were finally purified with Centricon-100.

The DNA was then quantified by means of both agarose gel electrophoresis (0.8%) stained with ethidium bromide and UV Spectrophotometry (Beckman DU 650).

We amplified 3 STR systems: HUMTH01 (Polymeropoulos M.H. et al., 1991), HUMVWFA31 (Kimpton C. et al., 1992), HUMBFXIII (Nishimura D.Y. et al., 1992); one AMP-FLP: D1S80 (Budowle B.J. et al., 1991); the HLA-DQα sequences and the 5 different loci of the Amplitype^{R} PM system (LDLR, GYPA, HBGG, D7S8, GC).

PCR protocols were carried out on a Perkin-Elmer 9600 Thermal Cycler according to the literature for D1S80 and as suggested by Promega (Geneprint^{TM} STR System) for HUMVWFA31, HUMTH01 and HUMBFXIII and by Perkin-Elmer (AmpliType^{R} HLA-DQα and AmpliType^{R} PM) for HLA-DQα and Polymarker loci. The typing of PCR products was achieved by horizontal polyacrylamide gel electrophoresis with silver staining for D1S80 and STRs and by means of reverse dot blot procedure as concern HLA-DQα and Polymarker.

Results and discussion

The observation with light microscope and SEM showed the presence of hair debris and epidermal cells. The aspect of the debris changes according to the different electric razors used. Rare nucleated cells are always present. These cells could explain the outcome that we obtained in extraction, amplification and typing of DNA. The successful typing percentage changes from 60% to 73% according to the different loci. We didn't note a significatively different rate about positive results from blades and electric razors (respectively 65% and 69%).

The results we obtained are summerized in the table, that shows the relatively successful typing rate, according to the systems used and the kind of specimens (razor or blade). We think this method could be used for forensic purposes in identification casework.

Table 1

Successful typing rate

Locus	electric razors (samples typed: **61**)	blades (samples typed: **66**)
HUMVWFA31	**70 %**	**70 %**
HUMBFXIII	**60 %**	**60 %**
HUMTH01	**73 %**	**70 %**
D1S80	**67 %**	**70 %**
HLA-DQα	**70 %**	**60 %**
Polymarker system	**70 %**	**60 %**

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