DNA TYPING ON SINGLE HAIR - RECENT POSSIBILITIES BASED ON NEW EXTRACTION METHOD

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INTRODUCTION

The analysis of hair is becoming increasingly important in the field of forensic science. It often represents the only element connecting the assailant to the victim, sometimes the only signature of crime, especially in cases of brutal crimes like kidnappings, murders, rapes. Usually, these evidences are collected on the crime scene or are found stuck on various surfaces (hood-caps, cushions, nails, etc.) but they are very limited in number, often only one sample.
The use of PCR and VNTR's as well as the most recent STR's analysis, have considerably increased the possibilities of success to the extent that in a matter of a few years we have gone from the limited methods based exclusively on the morphological study of hair, to the possible identification of the suspect, even starting with just one fiber.
The research in this field is very rich and interesting, but in most of the cases, freshly plucked hair samples were analyzed without taking into account that hair coming from crime scenes are usually shed hair in the telogen phase and, as is known, hardly allow to achieve DNA typing results. This is due to the fact that most of the DNA wich is located in the root and surrounding sheath cells is very poor in quantity and is usually less than 10 ng. Hence, among the various steps characterizing the analysis of DNA polymorphisms, the extraction method is certainly the most important, owing to the success of a test. The use of chelex ion-exchange resin, though very pratical, quick and particulary effective in the case of bloodstains, does not seem to be suited for DNA extraction from shed hair, as confirmed by the huge amount of scientific literature and by our experience.

MATERIALS AND METHODS

DNA EXTRACTION

Chelex Extraction

Telogen hair were separated into root portions and placed into a 1.5 ml microcentrifuge tube with 1 ml of sterile water. After an overnight incubation and centrifugation in a microcentrifuge 157.MP (OLE DICH), 200 μl of chelex solution (Chelex 100. Biorad Richmond, CA: 5% w/v in sterile water) were added. The following steps proceeded the indications of chelex protocols.

Single Tube Extraction

Telogen hair were separated into root portion and placed into a 2 ml microcentrifuge tube where an aliquot of a yellow resin portion (DNA SINGLE TUBE - BIOTECX Houston, Texas cat.NO.BL5500) had previously been added. The DNA sample was digested with proteinase K and then treated with phenol-chloroform mixture in the same tube during centrifugation, the polymer gel moves between the organic and acqueous phases to form an impenetrable barrier.

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The following steps of extraction proceeded according to the standard phenol-chloroform extraction protocol and the instructions given by Biotecx Bullettin NO. 8,1990. The method allows to limit the presence of inhibitors mainly represented by digested proteins.

AMPLIFICATION

Amplification and typing of the HLA-DQ alpha locus.

DNA was amplified by PCR using the Amplitype HLA-DQ alfa Forensic DNA Amplification and Typing Kit (Cetus Corporation,Emeryville, CA).

Amplification and typing of the VNTR's loci D1S80 and Apo B

Amplification of the VNTR's loci D1S80 and Apo B were achieved using the methods described previously by Budowle et al. and Boerwinkle et al. respectively.
The electrophoretical separation of the PCR products was performed using a high resolution horizontal rehydratable polyacrylamide gel as described by Budowle et al.. The gel was exposed through silver stain.

RESULTS AND DISCUSSION

Our experiment starts from some data taken from researches which investigate the possibility of extracting DNA from a telogen hair root. In particular, the Criminal Laboratory Department of Illinois presented a very interesting case history from Chelex extracts.
Considering the very good results obtained through Single Tube extraction on various types of biological traces, our aim was to test its effectiveness from telogen hair.
In the last six months, our lab has analyzed about 100 telogen hair, always using a Single Tube extraction.
The first twenty attempts to amplify the HLA-DQα locus, according to the P.E. protocol, either produced negative results or they were slightly visible dots. Then, we tried to re-amplify the DNA amplification fragments with a yield of two amplified products out of ten.
Both, however, showed weak non-specific dots after the hybridization.
Further experimental tests showed that this method was not the correct one, since the probability of obtaining not specific products proved to be quite high, especially if during the first amplification the maximum extract allowed was loaded.
Changing both the quantity of amplified DNA to be loaded on the reamplification and the number of cycles of re-amplification, no considerable improvements were obtained.
We tried to improve the results by adding BSA to the amplification mix.
The addition of BSA quantities equal to eight or sixteen micrograms in 50 microliters of final volume of mix did not produce satisfactory results (8 tests were performed). Moreover, if the amplification was performed without BSA simultaneously, the amplified DNA was undoubtedly better.
We obtained a higher quality in the typing of the HLA-DQα locus for hair in the telogen phase, when we carried out a Hot-Start on the amplification mix (25 μl mix, 25 μl $MgCl_2$, 20 μl of DNA extract, 95°C for 3 min., 2.5 U Taq Polymerase, amplification according to P.E. protocol).
From the 63 Single Tube extractions carried out on telogen hair,the majority resulting from forensic cases (ski hats, sweaters, foam rubber mattresses, etc.) only eight were unsuccessful, even if in thirty-three cases we obtained weak non-specific results in reverse Dot-Blot strips.

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These data permits us to affirm that the Single Tube extraction together with Hot-Start give us a high-success rate. However, a relatively low percentage (35%) of these amplified DNA is useful for identification, considering the high probability of non-specific dots.
Taking a high number of samples with non-specific results into consideration, we tried to transfer our experience reached on HLA DQα to other more frequently utilized loci in forensic biology (D1S80 and Apo B) to confirm the results obtained.
This was necessary so as to resolve some real cases, where the discrimination capability of HLA was not enough to show what exactly had occurred.
Starting from telogen hair and always using Single Tube protocol extraction together with Hot Start, we obtained a positive typing in each case either for D1S80 locus or for Apo B.
Although our data are not complete, we can certainly declare that an aimed amplification protocol allows us to obtain genotypes useful in identification, even if the evidence consists of only a shed human hair in the telogen phase.

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