PCR AMPLIFICATION OF DNA FROM OLD BLOOD STAINS

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Introduction

DNA typing from old bloodstains often gives inconclusive results, due to high degradation, as well as several inhibitors and enviromental factors that play a negative role on DNA extraction and amplification.
In order to better the results the Authors have applied the method of "Hot Start"by Mullis and others (1991), according to the technique suggested by Perkin Elmer Cetus.

Materials and Metods

In the manual Hot Start method, all reagents except Amplitaq DNA polymerase are mixed at room temperature though AmpliWax PCR Gems that procedure greatly facilitate a decrease the potential for contaminations.
This procedure involves dispensing one AmpliWax PCR Gems into a tube containing all components of the GeneAmp PCR process with the exception of DNA template, AmpliTaq DNA Polymerase, and buffer, heating and cooling the tube to melt the AmpliWax PCR Gem, thereby creating a solid wax layer above a lower reagent mix.
In the first denaturation step of the GeneAmp PCR process, the wax layer melts, the upper reagent mix thoroughly mixes with the lower reagent mix, and the DNA is denatured (see figure).
The basis principle of hot start PCR is the mixing of DNA and reagents only when both components are at high temperature. This maneuver prevents any interaction of primer and enzyme with DNA templates at low temperatures where specificity of annealing is not stringent. The original protocol has been slightly modified adding BSA (4 μgr/μl) to improve its efficiency.
In this case it was possible to perform the HLA DQα locus amplification after the completely negative results obtained through Chelex extraction.

Results

The characterization of genetic variation at the DNA level has generated significant advances in the forensic identification of individuals. A more flexible approach for individual identification at the DNA level is to use PCR. Specificity PCR provides a technique that is able to analyze small minute amounts of DNA from varios sources (1), including impure or degradated samples (2). DNA analysis from ten years old bloodstains was performed before by standard PCR, and then with HOT START Method (with BSA) (3). Inizially higly degraded human DNA produced negative results (Fig. 1).
In order to increase the likelihood of successfull amplification by PCR and to reduce the risk of contamination, we developed a HOT START Method with BSA. In this case dry and old bloodstains produce positive results.

The result obtained seems very interesting and represent a valid aid which can certainly contribute to the solution of a crime, even if we want to chek them in the near future through other marker and expand them with new samples.

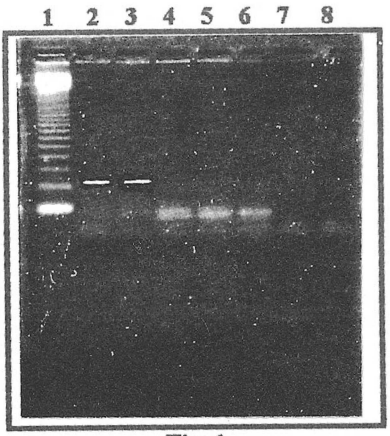

Fig. 1

Lane 1	**Ladder 123**
Lane 2	**PCR Control HLA DQα Locus**
Lane 3,4,5,6,7,8	**PCR products (10 ngr DNA) from bloodstains ten years old without BSA and Hot Start method.**

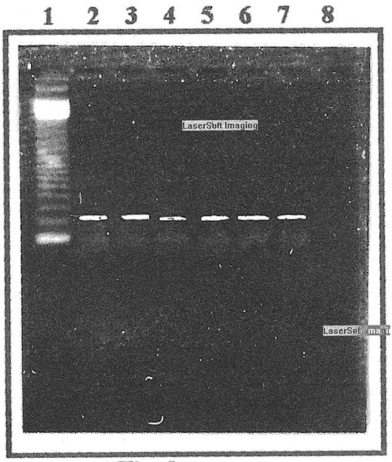

Fig. 2

Lane 1	**Ladder 123**
Lane 2	**PCR Control HLA DQα Locus**
Lane 3,4,5,6,7,8	**Like in Fig.1 but with BSA and Hot Start method.**

Reference

1) Higuchi R., Von Beroldingen C.H., Sensabaugh G.F. and Erlich H.A. (1988) "DNA typing from single hairs". Nature 332: 543 - 546.
2) Hagelberg E., Gray I.C. and Jeffreys A.J. (1991) "Identification of the skeletal remains of a murder victim by DNA analysis". Nature 352: 427 - 429".
3) Perkin Elmer , Amplification - A forum for PCR users - (July 1992): Vol. 8.