A SIMPLE METHOD TO PREVENT INHIBITION OF TAQ POLYMERASE AND HINFI RESTRICTION ENZYME IN DNA ANALYSIS OF STAIN MATERIAL

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Introduction

In forensic science case work DNA analysis of restriction fragment length polymorphisms (RFLP's) and amplified fragment length polymorphisms (AmpFLP's) have become the most powerful methods. But when working with different stain material (i.e. bloodstains, semenstains or vaginal secretions) which may be found on a variety of clothing and other objects, these carriers may interfere with the extraction and subsequent purification of DNA. In some of these cases this prevents restriction and/or amplification. Especially when analyzing cigarette tips restriction with HinfI and amplification with Taq polymerase may be inhibited. The addition of bovine serum albumin (BSA - Serva, Heidelberg) to the reaction mixture can decrease or overcome these inhibition problems.

Methods

DNA extraction and purification was carried out by standard protocols using Phenol/Chloroform reagents. Restriction of DNA was done with restriction enzyme HinfI (Boehringer, Mannheim). Amplification of Apo B 3'-Polymorphism (Boerwinkle et al., Schnee and Pflug) was carried out using 40 pmol of primers, 0.2 mM dNTP's in reaction buffer (10 mM Tris/HCl, pH 8.4), 2.5 mM $MgCl_2$, 50 mM KCl and a final volume of 50 μl, Taq Polymerase (2 U/reaction) came from Cetus Perkin Elmer. After an initial denaturation step of 2 min 27 cycles were carried out: 90 sec denaturation at 94° C, 90 sec annealing of the primers at 61° C, 2 min chain extension at 72° C. Amplified fragments were separated in 2 % agarose at 4 V/cm for about 7 hours.

Results

In our preliminary tests we added an equal amount (about 500 ng) of the same control DNA to each proteinase-K-digest for standardisation. Thus we got equal and good visible amounts of DNA after electrophoresis and staining with Ethidium bromide. **Prior** to the restriction an aliquot (1/10 volume) was taken from each DNA extract for the amplification of the Apo B 3'-region. Each aliquot was first split into two samples comprising one part and 9 parts respectively for parallel amplification. The total amount of DNA for ampflification was therefore about 45 ng and 5 ng respectively. The results show, that BSA can completely avoid inhibition of HinfI restriction enzyme. Dialysis of the DNA prior to restriction had no positive effect but led to a loss of DNA (Fig. 1A). Comparable results can be demonstrated for Taq Polymerase (Fig. 1B).

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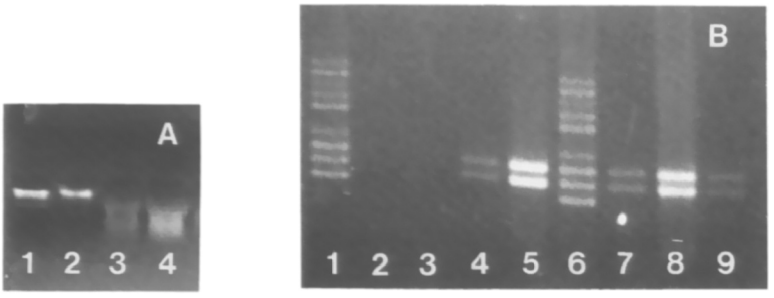

Fig. 1: Control DNA (500 ng aliquots) mixed with proteinase-K digest of cigarette tips and subsequent:

A restriction with HinfI: -DNA (1); standard restriction assay (2); standard assay + dialysis +0,1 % BSA (3); standard assay + 0,1 % BSA (4)

B Amplification of Apo B fragments: marker (1,6); standard amplification assay containing 5 ng and 45 ng of control-DNA respectively (2,3); standard assay + dialysis + 0,1 % BSA (4,5); standard assay + 0,1 % BSA (7,8); pure control-DNA (9)

The addition of BSA to the DNA extract prevents inhibition and gives a clearly visible band pattern of Apo B-fragments. Without BSA no amplification occurs. The effect of BSA takes place within a concentration of 0,5 % - 0,005 %.

In addition, Fig. 2, shows the amplified Apo B fragments of DNA extracted from cigarette tips used by different individuals. The DNA extract was divided into two parts and 0,1 % BSA was added to one part.
As shown in Fig. 2 only the reaction mixture supplemented with BSA gives a clearly visible band pattern.

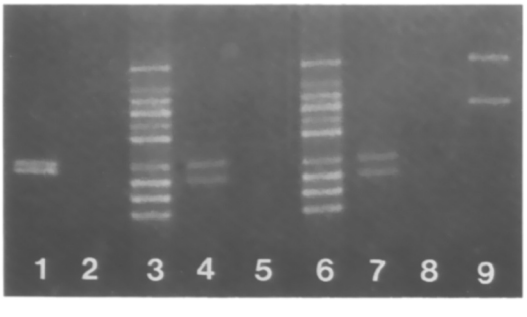

Fig. 2: Amplification of DNA extracted from cigarette tips smoked by different individuals: with 0,1 % BSA (1, 4, 7) and without BSA (2, 5, 8); marker consisting of a mixture of different Apo B fragments (3, 6); pos. control (9)

We often got comparable results regarding inhibition effects of Taq Polymerase with DNA extracts coming from vaginal secretions (supernatants of semen/vaginal secretion mixtures) and sometimes with bloodstains from dirty clothing or other objects. By adding BSA we were able to overcome

these inhibition effects. Fig. 3 shows the PCR results of some bloodstains. The amplification mixtures without BSA do not show any signals even on x-ray films after blotting and subsequent hybridisation with non-radioactive chemiluminescence labeled probe.

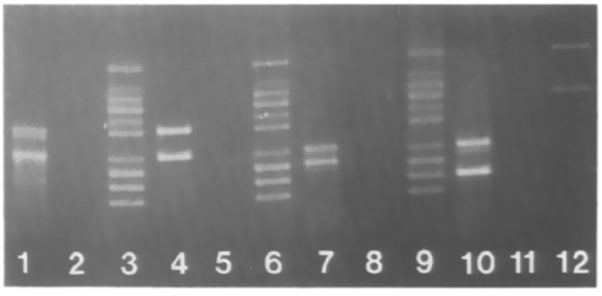

Fig. 3: Amplification of DNA extracted from bloodstains: aliquots supplemented with 0,1 % BSA (1, 4, 7, 10) and without BSA (2, 5, 8, 11) were analyzed; marker (3, 6, 9); pos. control (12)

References

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