PCR ON REUSABLE MATRIX-FIXED DNA

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INTRODUCTION

In forensic science case work DNA analysis has become the most powerful method. In the future the use of amplified fragment length polymorphisms like AmpFLPs (Boerwinkle et al., Kasai et al.) and STRs (Edwards et al., Polymeropolous et al.) will be mainly used in forensic stain analysis. But in contrast to the RFLP analysis with its high discriminating power the PCR method needs more than four or five different systems for individualization. In many cases when small stains of blood or other specimen have to be analyzed the amount of isolated DNA is only sufficient for the amplification of one or a few different PCR systems. On the other hand coamplification of several PCR systems (multiplex PCR) often does not lead to satisfactory results because optimal conditions for each system cannot be realized. Therefore we tried a different approach by using matrix-fixed DNA for repeated amplification of different PCR systems.

MATERIALS AND METHODS

Aliquots (1 - 2 μl) containing 50 ng, 5 ng and 500 pg of human DNA respectively were fixed on a immobilizing membrane. After cutting out these dots, remaining free charges were blocked with 1 % blocking solution (Boehringer, Mannheim) for about 1 h. The dots were then used for successive amplification of three PCR systems as shown in Fig. 1.

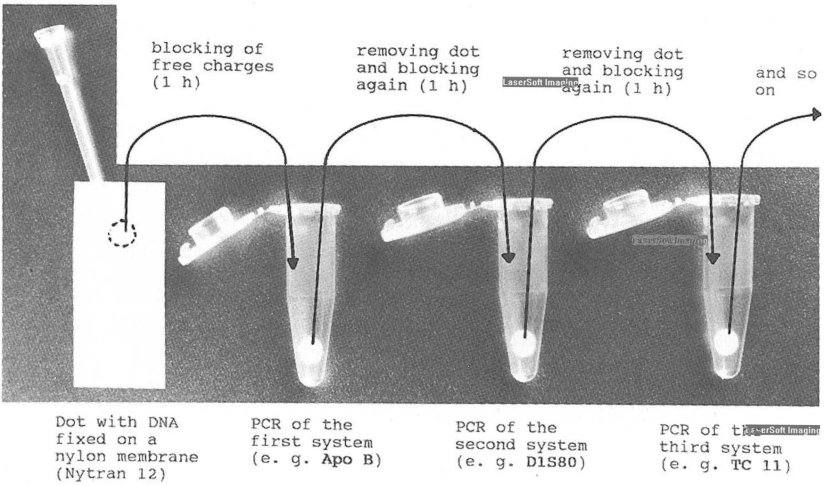

Fig. 1: Principle of repeated PCR with matrix-fixed DNA

In our experiments Apo B was amplified in a final volume of 50 µl containing 40 pmol of each primer, 200 µM dNTPs, 0,1 % BSA (Pflug et al.), the reaction buffer (10 mM Tris/HCl pH 8.4, 2.5 mM $MgCl_2$, 50 mM KCl) and 2 U Taq-Polymerase. 27 cycles with the following steps were carried out: denaturation 90 sec at 94°C, primer annealing 90 sec at 61°C and chain extension 120 sec at 72°C.
Amplification of D1S80 was carried out with the Forensic DNA Amplification Reagent kit from Perkin-Elmer according to the instruction of the manufacturer. TC 11 (HUMTH01) was amplified according to the conditions described for Apo B except the cycling parameters: denaturation 45 sec at 94°C, primer annealing 30 sec at 60°C and chain extension 30 sec at 72°C, 32 cycles.
Electrophoresis of Apo B and D1S80 products was performed on 1,5 % agarose gels with subsequent blotting and hybridization. As probes we used homologous DNA fragments amplified in the presence of Dig-UTP (Boehringer, Mannheim). Detection of the PCR fragments was carried out by chemiluminescence on x-ray films. TC 11 products were separated on polyacrylamide gels and silver stained.

RESULTS AND DISCUSSION

In our tests we started PCR with the AmpFLP system Apo B followed by D1S80 and the STR system TC 11. Changing the succession didn't affect the results significantly. Among the different membranes we tested (Biodyne B, Pall, Nytran 12 and NC, Schleicher u. Schüll) Nytran 12 seems to be superior. If the dots remained in the reaction cap during the whole amplification procedure we normally could detect amplification products only at the highest level of 50 ng of DNA in the case of Apo B and D1S80 and at 50 ng and 5 ng in the case of TC 11. Better results were obtained doing a preamplification of 5 - 11 cycles with 1 : 40 decreased amounts of primers and nucleotides. Then we started a new amplification cycle without the dots but with an aliquot of 5 - 10 µl of the preamplification mixture. Under these conditions we got PCR products even with the 5 ng dots in case of Apo B and even with the 500 pg dots in case of TC 11 as it is shown in Figure 2. The consistently better efficiency of the amplification of the TC 11 system is probably due to its much shorter fragments compared to Apo B and D1S80.
Our results show that successive amplification of three different PCR systems on the same matrix-fixed DNA is possible. This offers the possibility to do several PCR systems even with very small amounts of DNA.

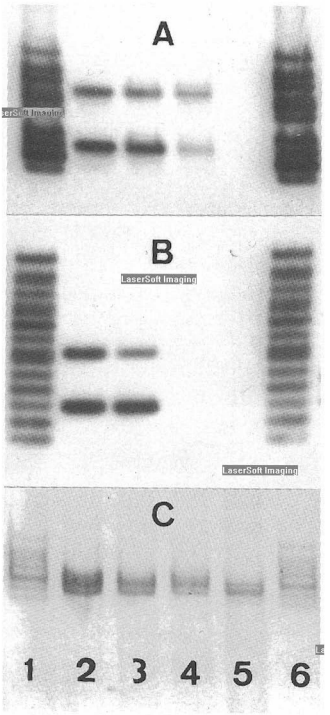

Fig. 2:

Successive amplification of Apo B (A), D1S80 (B) and TC 11 (C) fragments on nylon-fixed DNA: marker consisting of a mixture of different allele fragments (1, 6), control DNA (2), dots with 50 ng (3), 5 ng (4) and 500 pg (5) of the same DNA fixed on a nylon membrane.

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