POLYMERASE CHAIN REACTION: TYPING OF DNA ISOLATED FROM VARIOUS FORMS OF BIOLOGICAL EVIDENCE

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SUMMARY

The polymerase chain reaction (PCR) is a promising technique for forensic case work because it allows the analysis of very small stains. One of the best characterized marker systems for PCR is the apo B 3' length polymorphism. We show here typing results from various forms of biological evidence and demonstrate the application of the PCR-technique in forensic case work.

INTRODUCTION

After the introduction of the RFLP analysis into forensic science the possibilities of analysing various forms of biological evidence have further been extended by the PCR. Several PCR typing systems based on length or sequence polymorphisms have been described. One of the most polymorphic systems is the apo B 3' length polymorphism on chromosome 2 about 500 bp 3' to the last codon of the apolipoprotein B gene (Knott 1986, Boerwinkle 1989). It consists of a variable number of tandem repeats, each 14-16 bp long. In Germany 14 alleles from 580 to 910 bp have been found with frequencies ranging from about 0.2 % to 37 %.
We chose the apo B polymorphism as the first PCR typing system for our case work. Some examples of DNA analysis from various stains are shown here.

MATERIAL AND METHODS

The DNA was isolated with standard methods using phenol/chloroform extraction. Mixtures of vaginal secretion and semen were separated by differential cell lysis prior to extraction. Once resuspended in TE buffer the DNA was divided into 2 or 3 aliquots containing different amounts of DNA. Each sample was amplified in a final volume of 50 ul containing 40 pmol of each primer (5' TGG AAA CGG AGA AAT TAT GGA GG 3', 5' CCT TCT CAC TTG GCA AAT ACA ATT 3'), 0.2 mM dNTPs, 2 U Taq-Polymerase and the reaction buffer (10 mM Tris/HCl pH 8.4, 2.5 mM MgCl , 50 mM KCl). After an initial denaturation step of 2 min, 27 cycles with the following steps were carried out: denaturation 90 sec at 94° C, annealing of the primers 90 sec at 61° C, chain extension 120 sec at 72° C. Electrophoresis of the amplified products was performed on 2 % agarose gels at 4 V/cm for 6-7 hours.

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RESULTS

We have analysed DNA from rape cases, i.e. DNA derived from sperm and/or vaginal secretion, as well as bloodstains or hair roots from murder cases or robberies. While sperm usually did not cause problems, DNA from vaginal secretion sometimes failed to amplify. We do not know whether this might be due to inhibitors or to large amounts of degraded DNA but we observed that amplifying DNA in several different dilutions steps (e.g. 1:1 and 1:10 or 1:1, 1:5 and 1:25) and/or adding BSA to the amplification mix (see Pflug et al., poster) usually leads to better results. When there were enough cells, DNA isolated from hair roots normally amplified well though the amount of amplified product did not always correlate with the number of cells estimated microscopically.

An example for PCR analysis in a rape case is demonstrated in Fig. 1. Suspect B reveals the same alleles as the stain (sperm) whereas the pattern of the victim is different. The frequency of the allele combination of the stain was in this case 0.197. This is the most common genotype in Germany. All other genotypes have frequencies of less than 0.129.

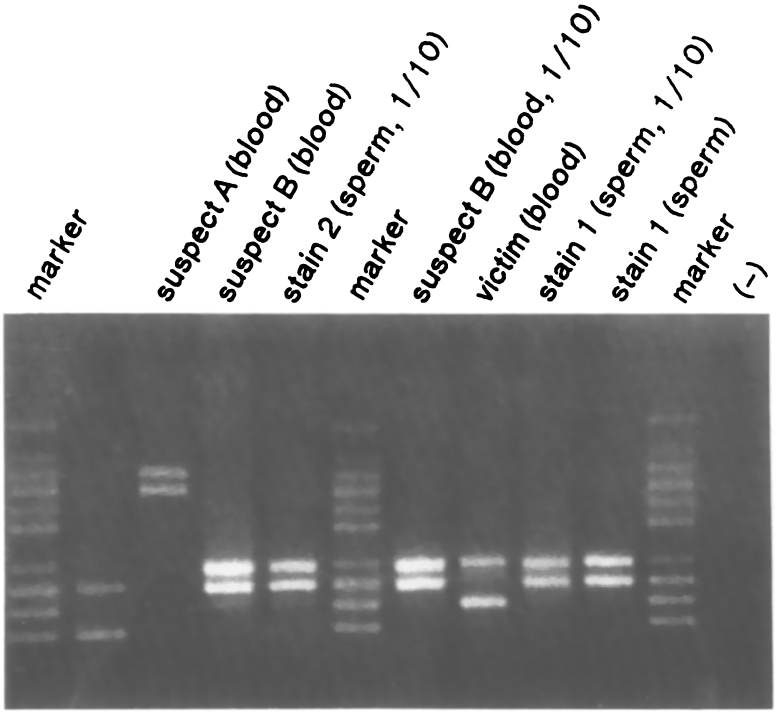

Fig. 1: apo B typing results of a rape case
(-): negative control, containing all ingredients of the amplification mix except for a DNA template,
the marker is a mixture of 10 different alleles from several individuals

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DISCUSSION

The apo B 3' length polymorphism provides several advantages: it is one of the most polymorphic loci suitable for PCR, it is well characterized, the alleles are short and can therefore be amplified easily. As they differ in length blotting and hybridization is not necessary.
To get optimal results in case work we find it useful to proceed in the following manner: first we usually amplify two or three different dilutions of the DNA isolated from a stain or from blood because we observed that when one amplification was not successful a higher or lower amount of DNA often amplified well. Before running the main gel we first control the success of the amplification reaction with an aliquot (usually 1/5 of the volume). This allows us to chose the best amplification of the stain and place it between or next to the DNA of the suspect and the victim. Instead of a bacterial or viral size marker we place a ladder of 8-10 different known alleles at least every fifth slot so that determination of the unknown alleles is very easy. For frequency calculation of the allele combination we use our own statistical data which comprise about 340 German individuals up to now and which correspond well to data from other ethnic groups.
Compared to RFLP analysis typing of amplified fragment length polymorphisms is very rapid. If there are a lot of suspects which cannot be excluded by conventional methods their number can be decreased by PCR before performing RFLP analysis. Therefore, typing by PCR is also a good screening method to save time and money.

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