The approach of using random priming for small forensic DNA-samples

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Introduction

We are investigating the applicability of a new method called primer-extension-preamplification (PEP; Zhang et al., 1992) for forensic casework. Using standard PCR procedures the sensitivity for STR`s lies between 50-100 pg, and for AmpFLP`s between 500 pg - 1ng of genomic DNA. In the past DNA-samples containing very small amounts of template DNA could only be analyzed once. Employing PEP prior to the regular PCR we have been able to type several VNTR-loci from one PEP-sample. In order to systematically study the advantages and disadvantages of the PEP procedure, we amplified DNA-solutions in varying degrees of magnitude using random primers and analyzed aliquots of the PEP-reaction for various VNTR`s.

Materials and Methods

Several DNA-dilutions were amplified by multiple rounds of primer extension using a collection of 15-base oligonucleotides in which any one of the four possible bases could be present at each position.

Each PEP amplification sample contained:
1µl template (100pg, 50pg, 10pg), 1,5 µl of a 400µM solution of random primers (Operon Technologies, Alameda, CA), 3µl 10xPCR buffer, 1,5µl of a mixture of the 4 dNTPs (each at 2mM), 0,4µl Taq polymerase (5U/µl, Promega), brought to 30µl with water

Temperature cycle: denaturation for 1 min at 92°C, annealing for 2 min at 37°C, extension for 4 min at 55°C, a total of 40 or 50 cycles was carried out in a Trio-Thermoblock (Biometra)

Aliquots of the PEP were tested for specific DNA sequences.
Nested Apo B analysis was performed as described previously (Schmitt et al., 1994).

VWA/F13A1
Amplification conditions
2 U Taq polymerase (Promega), 0,25 µM each primer, 150 µM dNTPs, 2,5 µl 10x PCR buffer, 1,5 mM $MgCl_2$
Temperature cycle (VWA): 1 min 94°C, 1 min 50°C, 2 min 72°C, 29 cycles
Temperature cycle (F13A1): 1 min 94°C, 1 min 55°C, 2 min 72°C, 27 cycles

Primer sequences (VWA; Kimpton et al., 1992)

Primer sequences (F13A1, Polymeropoulos et al., 1991)

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Electrophoresis

Apo B electrophoresis was performed as described previously (Schmitt et al., 1994).

For VWA: Polyacrylamid gels (8% T, 3% C) were 0,8 mm thick with 1x TBE. The vertical electrophoresis was run for 26 cm (1200 V, 65 W, 90 mA) at 50°C - 60°C on the Gibco BRL Model S2 Electrophoresis Apparatus. The DNA fragments were detected by silver staining.

For F13A1: Long Ranger (5%), Urea (7M), gels were 0,35 mm thick with 1xTBE. The electrophoresis was run for 240 min (1500 V, 38 mA, 34W, 3 W Laser, at 40°C). The DNA fragments were visualized by fluorescence detection using the A.L.F. sequencer (Pharmacia).

Results

Our first experiment was designed to estimate the efficiency of the PEP procedure. DNA of three different individuals was diluted to achieve varied concentrations which were verified using the dot blot procedure.

Table 1: VWA typing results with and without the PEP procedure

Lane	Sample	Genotype	DNA amount	PEP	Results
2	215	17/19	100 pg	yes	17/19
3	215	17/19	100 pg	no	no result
4	215	17/19	10 pg	yes	19
5	215	17/19	10 pg	no	no result
7	216	16/17	10 pg	yes	16/17
8	216	16/17	10 pg	no	no result
9	217	14/15	10 pg	yes	14/15
10	217	14/15	10 pg	no	no result

The PEP procedure increased the sensitivity of VWA typing. Two 10 pg samples that couldn`t be typed without PEP yielded correct results. A strong preferential amplification of the longer allelic product could be observed.
In order to find out if the PEP reaction produces fragments of at least 600 bp in length or more the AmpFLP Apo B (allele range 590 - 900 bp) was typed for the PEP samples.

Correct ApoB genotypes were identified for 17 out of 36 reactions, which means that the PEP generates fragments >590 bp. The typing results shows a high incidence of no result or allelic drop out. Increasing the PEP aliquot input for ApoB PCR (5 µl, 10 µl, 20 µl; data not shown) did not improve the success rate.

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Table 2: ApoB typing results after PEP reaction for different individuals and DNA concentrations

Sample	Genotype	DNA amount for PEP	input after PEP for nested ApoB analysis	Total no. of investigations	Results
215	33/37	100 pg	1 µl	3	2x 33/37 1x 33
216	37/37	100 pg	1 µl	3	3x 37
217	35/37	100 pg	1 µl	3	3x 35/37
215	33/37	50 pg	1 µl	3	2x 33 1x artificial band
216	37/37	50 pg	1 µl	3	3x 37
217	35/37	50 pg	1 µl	3	2x 35/37 1x no result
215	33/37	10 pg	1 µl	6	1x 33 5x no result
216	37/37	10 pg	1 µl	6	4x 37 2x no result
217	35/37	10 pg	1 µl	6	6x no result

Table 3: F13A1 typing results after PEP procedure for different individuals and DNA concentrations

Probe	Genotype	DNA-amount for PEP	input after PEP for F13A1 analysis	Results
215 (lane 1)	6/7	50 pg	1 µl	no result
215 (lane 2)	6/7	50 pg	5 µl	6/7
216 (lane 3)	5/5	50 pg	1 µl	no result
216 (lane 4)	5/5	50 pg	5 µl	5
217	5/7	50 pg	1 µl	5/7
217	5/7	50 pg	5 µl	5/7
215 (lane 29)	6/7	100 pg	1 µl	6/(7)
215 (lane 30)	6/7	100 pg	5 µl	6/7
216 (lane 32)	5/5	100 pg	1 µl	5
216 (lane 33)	5/5	100 pg	5 µl	5
217	5/7	100 pg	1 µl	5/7
217	5/7	100 pg	5 µl	5/7

Since the detection limit for F13A1 in our hands lies between 50 pg - 100 pg the approach of PEP increased the sensitivity for this locus too. Here, increasing the DNA input after PEP from 1 µl to 5 µl improved the results, so that both alleles for each person could be reproducibly identified.

Discussion

Our results show that the PEP procedure enhances the sensitivity of PCR DNA typing. The most reproducible results could be obtained by using 100 pg amounts of DNA. Since only 1µl aliquots are amplified further, in optimal cases a 30µl PEP sample allows the typing of 30 loci, a number that even with multiplexing of primer pairs would be difficult to achieve otherwise. Aside from the results that are shown here, the loci HumTHO1 and SE33 could also be correctly typed after PEP, so that so far we were able to get results from five loci for one PEP sample starting with 100 pg of DNA.
The PEP results were influenced by allelic drop out, not only of the larger, but also of the smaller alleles. The occurence of allelic drop out was theoretically expected; allelic drop out of the smaller alleles was only observed for 10 pg amounts of DNA. DNA dilutions this low are affected by stochastic effects and random fluctuation of alleles. Parallel to specific PCR amplification the random primer extension is expected to be less effective for longer stretches of DNA. This expectation is verified by our ApoB results: the longer alleles were the first drop out; compared to the STR`s the necessary DNA input was higher. Since nested PCR has a single cell detection limit, the fact that even the amplification of PEP aliquots of 20µl didn`t improve the results, means that the ApoB alleles were not present in the PEP sample. The size distribution of PEP products is not known, and will be difficult to establish since the amount of DNA present after PEP is still to low to be detected by conventional means. Furthermore using haploid single cells, Zhang et al. (1992) showed that only 80% of the genomic DNA are amplified by the random primers, which leads to a statistical incidence of drop out of genetic loci.
Huber and Holtz (1994) described the succesful application of PEP for several forensic samples. Our study was designed to generate data regarding the reliability of PCR typing results after PEP. The occurence of preferential amplification and allelic drop out pose a major problem, because it could lead to the incorrect typing of a heterozygote sample as homozygote. PCR typing following PEP should therefore be limited to STR loci with short alleles. Even though results can be obtained with less DNA, for forensic case work, the PEP DNA input should not be lower than 100 pg to avoid any stochastic effects. Following these precautions, PEP offers the possibility to achieve reliable typing results for several loci with very small amounts of DNA.

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