RANDOM PRIMING AND MULTIPLEX PCR WITH THREE SHORT TANDEM REPEATS IN FORENSIC CASEWORKS

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INTRODUCTION

In our work we present two new tools for forensic caseworks. Firstly a multiplex PCR with three short tandem repeats (STRs): an easy and rapid method for individual identification with high probability. After optimization of the PCR conditions (especially temperature profile and primer concentrations) slippage bands are avoided and thereby the STRs are adapted to forensic demands. The PCR products of the chosen loci (characteristics see table 1) are simultanously detected on a single PAA-gel.
Secondly we demonstrate the usefulness of preamplification with random primers. The procedure, named Primer Extension Preamplification (PEP), was developed by Zhang et al. [4] for preimplantation purposes. Our studies show that PEP is helpful in case of hampered PCR caused by minute amount of template DNA.To our knowledge this is the first report of forensic application of PEP.

MULTIPLEX AMPLIFICATION OF THE STRs (fig. 1)

Table1

Locus	Chromosomal Location	Number of Alleles	Polymorphic Repeat	Range of Allele size
Human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP 2, SE 33)	Chromosome 6	26	$[AAAG]_n$	226-330 bp
Androgen receptor gene [2] (AR)	X-Chromosome	11	$[CAG]_n$	195-225 bp
Apolipoprotein C-II gene (Apo C II) [3]	Chromosome 19	15	$[CA]_n$	127-171 bp

CRITERIA FOR THE SELECTION OF THESE LOCI
- compatibility of the three systems in multiplex PCR
- high polymorphism
- balanced distribution
- non overlapping allele sizes

PCR-protocol:
1-10 ng template DNA, 200 µM each nucleotide, 0.2 µM each primer- APO C II, 0.4 µM each primer-Androgen receptor gene, 1 µM each primer-ACTBP 2, 2.5 µl 10x buffer (Boehringer) diluted to a total volume of 23 µl with distilled water.
5 min Hot Start at 94 °C, 0.8 U Taq polymerase (Boehringer), diluted in 2 µl water, were added after the Hot Start denaturation (at 80 °C).
Cycling parameters:
denaturation 1 min 94 °C, annealing 1.5 min 64 °C, extension 1.5 min 72 °C, 33 cycles final extension 10 min 72 °C

RANDOM PRIMING - PRIMER EXTENSION PREAMPLIFICATION (PEP)

Definition:

PEP is a PCR with an ensemble of 15-mer random oligonucleotides, in which anyone of the four possible bases could be present at each position. The random primers are maximally composed of a mixture of 4^{15} sequences. The probability of amplifying any sequence in the genome to a minimum of 30 copies is not less than 0.78.
After pretreatment with PEP an aliquot of the reaction mixture is used as template in a single locus-specific PCR. A multiplex PCR with the PEP-product is not possible.

Collection of samples, which are typable only after PEP:

- lung tissue, stored one year in non-buffered formalin

- chelex-extract of a cigarette butt (fig. 2)

- diluted DNA-extracts derived from blood and sperm samples (10-20 pg DNA)

- morphologically sorted single sperms

Different extraction-procedures and modified locus-specific PCR without PEP do not yield PCR products.

PCR-based systems tested after preamplification with random primers:
PCR suceeded: HLA-DQ A1, ACTBP 2, APO C II, CTLA 4, HLA CLASS I fragment (620 bp) [5]
PCR not suceeded: D1 S80, Tri-repeat at the androgen receptor gene

PCR protocol and cycling parameters of the PEP-reaction are described elsewhere [4]

RESULTS and DISCUSSION

Our multiplex PCR has two advantages, which will be helpful in routine caseworks:
1. the discrimination power achieved in one working process is very high (probability, calculated with maximal allele frequencies, is lower than 1 in 10.000)
2. the distinct bands are detected by silver staining; fluorescence- or radioactive labelling is not required
An optimization of the PCR-conditions seems to be much more important for multiplex PCR than for a single one.
Until extensive mutation studies are not available, the system should be used preferably for identity testing. We find one mutation at the androgen receptor gene locus in a panel of 20 mother,child,father-trios.
Our experiences with random priming demonstrate the usefulness in case of otherwise unamplifiable template DNA. "PEP-supported" typing of formalin fixed tissue could be applicated for clinical purposes too. Forensic issues concerning mixed samples may be solved by sorting cells morphologically and use them as substrate in a PEP-reaction followed by some locus-specific PCR.
This procedure allows to generate enough template DNA for several PCR-reactions in case of limited DNA-amount (< 1 ng DNA).
An amplification of PCR-products up to a size of 620 bp is possible.
Allelic drop out or mutation caused by the PEP-treatment are not observed.

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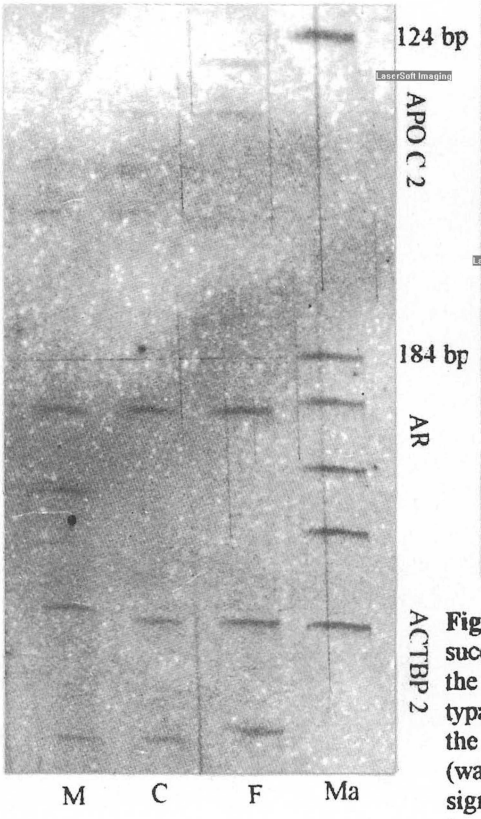

Fig. 1
A mother (M)- child (C)-father (F)-trio
sex of child: male
Ma: marker

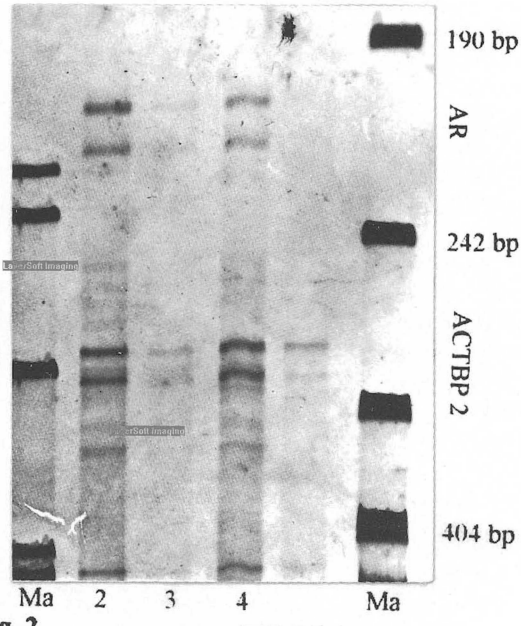

Fig. 2
successful application of random priming: the chelex extract of a cigarette butt (lane 5)was typable after PEP. The extract was prepared from the outer paper of the filter. Two negative controls (water and extract of the filter inlay) gave no signal after treatment with PEP.
lane 2-4: hair, blood and a bone fragment of the victim involved in the same case
Ma: marker
The samples shown here are typed with a duplex PCR (except the PEP product)