

TRIPLEX PCR OF THREE STR LOCI WITH NON-OVERLAPPING ALLELE SIZES

I. Rostedt and M. Lukka

National Public Health Institute, Helsinki, Finland

INTRODUCTION

One of the advantages in the PCR methodology is the possibility to amplify simultaneously several loci (multiplex PCR) which improves remarkably the information obtained from one analysis. Here we describe a triplex PCR of three STR loci: HumTPO (Anker 1992) in chromosome 2, HumTH01 (Edwards 1991) in chromosome 11 and D3S1359 (Li 1993) in chromosome 3.

With suitable primers the allele size ranges in the HumTPO (106-130 base pairs; bp), HumTH01 (155-179 bp) and D3S1359 (197-265 bp) loci do not overlap which made it possible to amplify the loci simultaneously using unlabeled primers and perform the size separation and allele detection using electrophoresis and silver staining without expensive automation.

MATERIALS AND METHODS

Sample preparation: DNA was extracted from 3 μ l blood samples with the Chelex (R) resin-extraction approach (Walsh 1991). One μ l from the 200 μ l extract was used for triplex PCR amplification.

PCR-amplifications:

Locus	Primer sequences	Concentrations
HumTPO	5' CAC TAG CAC CCA GAA CCG TC 3'	0.40 μ M
	5' CCT TGT CAG CGT TTA TTT GCC 3'	0.40 μ M
HumTH01	5' GTG ATT CCC ATT GGC CTG TTC CTC 3'	0.45 μ M
	5' GTG GGC TGA AAA GCT CCC GAT TAT 3'	0.45 μ M
D3S1359	5' ATG CTA AGT GCT AAG TCA ACT 3'	1.40 μ M
	5' GTT GCC TCT GAC ATG GCT TT 3'	1.40 μ M

In PCR-amplifications the nucleotide (dNTPs) concentration was 0.2 mM in a PCR buffer consisting of 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton x-100. The PCR was performed in 100 μ l volume under a layer of mineral oil using MJ-Research PTC-100 thermal cycler.

Each amplification was initiated with a hot start (Chou 1992) and 2.5 U Dynazyme-polymerase (Finnzymes, Finland) were used as DNA-polymerase.

Amplification program was as follows:

94°C 1', 55°C 45'', 72°C 45''; 32 cycles.

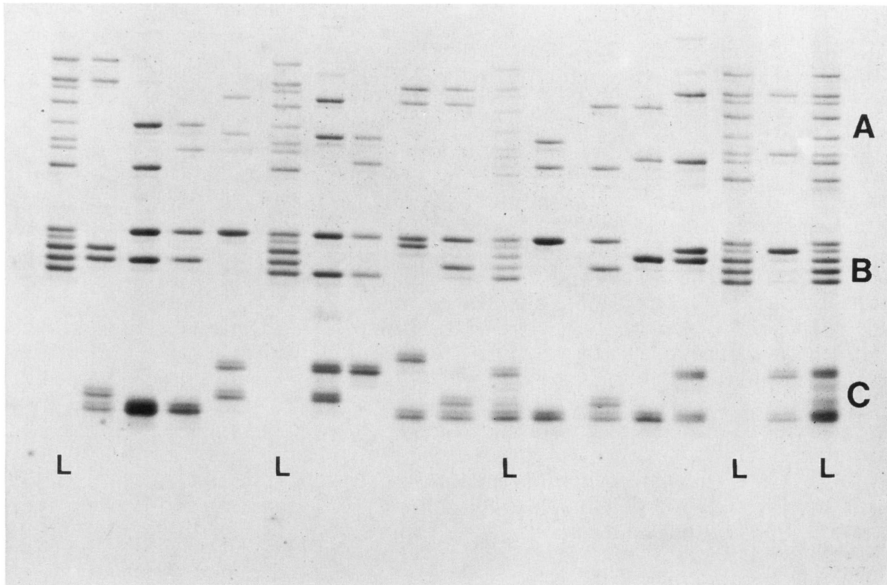
Electrophoresis: A 10 μ l aliquot of PCR-product containing 2 μ l of sample buffer was applied for electrophoresis in vertical PAGE (Sajantila 1993).

The separation gel 12 x 14 x 0.075 cm (10% T, 4.8% C) and the stacking gel 4 x 14 x 0.075 cm (3.0% T, 4.8% C) were made from acrylamide stock solution 30% T, 4.8% C containing 21% glycerol in the gel buffer (33mM Tris-sulfate, pH 4.5).

The electrophoresis was carried out for 17 hours at 200 V in +4°C using 100 mM Tris, 100 mM boric acid, 2 mM EDTA; pH 8.5 as the running buffer.

The amplified fragments were visualised by silver staining (Allen 1989) and the gels were dried for documentation.

RESULTS AND DISCUSSION



Silver stained patterns at the HumTPO (C), HumTH01 (B) and D3S1359 (A) loci after triplex PCR and PAGE. L = allelic ladder containing alleles 8 - 11 for the HumTPO locus, alleles 6 - 10 (9.3) for the HumTH01 locus and alleles 2, 4, 5, 7, 10, 12, 13 and 16 for the D3S1359 locus. The allele nomenclature for D3S1359 is tentative.

The HumTPO, HumTH01 and D3S1359 loci can be safely genotyped simultaneously utilizing the triplex PCR method. No discrepancies has been found between the results after single or triplex PCR.

In the Finnish population the mean exclusion probabilities (Gürtler 1956) of HumTPO, HumTH01 and D3S1359 loci in paternity testing are 33.4%, 58.5%, 76.6% respectively. The combined exclusion probability of these three loci is 93.5%.

References:

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