

## A Tetraplex PCR system for the analysis of paternity cases

C.Seidl, O.Jäger, E.Seifried

Institute for Transfusion Medicine and Immunohematology, Red Cross Blood Donor Service Hesse, Sandhofstrasse 1, 60528 Frankfurt, Germany

### INTRODUCTION

Short tandem repeat loci (STR) with repeat unit length between 2-6 bp represent highly polymorphic markers in the human genome, that are ideal markers for genomic mapping and genetic linkage analysis and can be used for forensic and paternity applications. In contrast to the highly polymorphic VNTR loci, STR loci exhibit a limited polymorphism resulting in a reduced discrimination power. Feasibility of amplification of STR loci using the polymerase chain reaction allows to multiplex several STR loci in a single PCR reaction. In combination with the use of fluorescence labelled primers employing different fluorescence dyes STR loci with overlapping or identical allele size ranges can be analyzed together in the same lane of a polyacrylamid gel thus providing a rapid and sensitive method for human identification.

We have established a tetraplex PCR-STR system to combine the discrimination power of four tetraplex STR polymorphism at locus HUMTH01, HUMCYP19, HUMD8S639 and HUMACPP.

### METHODS AND MATERIALS

**STR-loci and PCR conditions.** The STR Loci and corresponding primer sequences are summarized in table 1.

Table 1: Primersequences and fluorescence dye labels of the tetraplex PCR system

STR-Loci	Primersequences 5'- 3'	Dye-label	Ref.
HUMTH01 locus: 11p15.5-p15 repeat: AATG	P5 GTGGGCTGAAAAGCTCCCATTAT P3 GTGATTCCCATTGGCTGTTCCTC	HEX	Polymeropoulos 1991
HUMCYP19 locus: 15q21.1 repeat: TTTA	P5 GCAGGTACTIONTAGTACTAC P3 TTACAGTGAGCCAAGGTCGT	FAM	Polymeropoulos 1992
HUMACPP locus: 3q21-qter repeat: AAAT	P5 GGGCAACATGGTGAAACCTT P3 CCTAGCCTATACTTCCTTTC	TAMRA	Polymeropoulos 1991
HUMD8S639 locus: 8p21-p11 repeat: AGAT	P5 GAGTGATGGAAGAAAACAAGTAGC P3 CTCAACCAAAAAATGTAAAGTCAGG	HEX	Nelson 1994

All oligonucleotides were commercially synthesized and the 5'Primers were labeled with fluorescence dye markers (ABI DNA Facility, Weiterstadt, Germany). Following the allelic size distribution of the different STR-Systems, HUMCYP19 was labelled with FAM (5-carboxylfluorescein), HUMTH01 was labelled with HEX (6-carboxy-2',4',7',4,7-Hexachlorofluorescein), HUMACPP was labelled with TAMRA (N,N,N',N'-tetramethyl-6-carboxyrhodamine) and HUMD8S639 with HEX. Tetraplex PCR amplification was performed using 10-100ng genomic DNA in a 50µl reaction volume comprising 0,5 Units Taq DNA polymerase (Appligene), 1x PCR buffer (Appligen), 200 mM each deoxyrinucleoside triphosphate

(dNTP) (Boehringer Mannheim, Germany). Samples were amplified for 29 cycles of 30 sec at 94 C, 40 sec at 54 C and 30 sec at 72 C followed by a 5 min extension period at 72 C on a Perkin Elmer 9600 DNA Thermal Cycler. Primer concentrations were 10 pmol D8S639/P5 and P3, 5 pmol ACPP/P5 and P3, 5 pmol TH01/P5 and P3 and 5pmol CYP19/P5 and P3.

**Allele size analysis.** PCR products were analysed together with an internal standard in the same lane of a standard 6% polyacrylamid denaturing sequencing gel. The internal standard fragments were generated by PCR amplification using various primer combinations from the vector pGL2-basic (Promega). Internal standard fragments were labelled with the dye ROX (6-carboxy-X-rhodamine). A 2,5 µl aliquot of each amplification reaction was combined with 6 fmoles of the internal standard. Subsequently, samples were heat denatured before being loaded. Fragment separation was performed by gel electrophoresis at 30 W for 8 hours on an Applied Biosystems automated DNA sequencer model 373A. Relative position of fragments were calculated by measuring the laserlight induced fluorescence emission of the DNA fragments. Fragment sizes were determined automatically using GENESCAN 672 software (Perkin Elmer-ABI) employing the SOUTHERN local method for local reziproc calculation of fragment sizes utilising standard fragments of known size. Alleles were assigned according to the number of repeat unit of the individual allele. Alleles of HUMTH01 were assigned according to published sequences (Edwards 1992) whereas for allele assignment of HUMCYP19, D8S639 and HUMACPP individual alleles that we observed in our population study were sequenced.

**Statistical analysis.** Allele and genotype frequencies were collected from a minimum of 100 unrelated caucasian individuals for each STR loci. The polymorphic information content (PIC) was calculated using the formula of Botstein (1980). The discrimination index (DI) and the matching probability (pM) were calculated by the method of Jones (1972). The sample gene diversity (frequency of heterozygotes expected under Hardy-Weinberg equilibrium) was calculated as discribed by Kimpton (1993). Hardy-Weinberg-Equilibrium was calculated using the Chi squared goodness of fit test.

## RESULTS

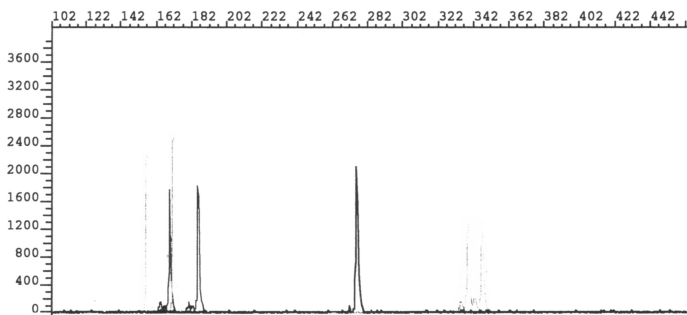


Fig.1: Fluorescence based analysis of the tetraplex STR system on a 6% polacryamid gel.

Table 2: Heterozygosity rate (HR), polymorphic information content (PIC), discrimination index (DI) and matching probability (pM) of the tetraplex PCR system.

STR loci	Alleles	Size range (bp)	HR	PIC	DI	pM
HUMTH01	6	154 - 174	0,746	0,727	0,91	0,09
HUMCYP19	7	168 - 191	0,727	0,719	0,88	0,12
HUMD8S639	14	317 - 373	0,857	0,819	0,95	0,05
HUMACPP	6	263 - 283	0,630	0,591	0,81	0,19
Combined					0,99	$1,0 \times 10^{-4}$

## DISCUSSION

In the present study we describe a tetraplex PCR system that combines STR loci with heterozygosity rates between 0,63 to 0,86 from four different chromosomes. All STR loci are easily amplified using the PCR conditions described giving distinct fragment sizes. Locus ACP, however, is sensible to generate unspecific amplification products. Thus we would recommend to use an individual fluorescence dye label for this locus in a multiplex PCR system. Sequence analysis of the loci HUMCYP19, HUMD8S639 and HUMACPP reveals a basic tetranucleotide repeat unit structure of the individual alleles. In the present population sample, we observed 7 alleles for Locus HUMCYP19, 6 alleles for the loci HUMTH01 and HUMACPP and 14 alleles for locus D8S639. Locus D8S639, however comprises alleles that have structural differences but identical fragment sizes. Analysis of these alleles can only be performed using allele groups.

The combined discrimination index of all four STR loci is 0,99 with a probability of match of  $1,0 \times 10^{-4}$ . Combined pM values of all analysed loci below  $10^{-8}$  may not be sufficient to determine paternity cases. The combination of this tetraplex system with additional STR loci and / or multiplex systems should however increase pM values and thus will contribute to improve the analysis of paternity or forensic cases.

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