

NON-AUTOMATIC MULTIPLEX ANALYSIS AND DETECTION OF SIX STR-LOCI: HPRT, FABP2, CD4, F13A1, CYP19 AND LPL

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SUMMARY

We present a DNA-typing method using six unlinked STR loci (HPRT, FABP2, CD4, F13A1, CYP19, LPL). All loci are separated in one gel, using one lane for three different loci. Electrophoretic analysis was non-automatically performed, followed by silverstaining in a native, vertical gel system.

INTRODUCTION

Personal identification by use of DNA typing methodologies has been an issue in the popular and scientific press. Many years of experience have demonstrated that DNA typing methodologies, using a battery of highly polymorphic RFLP-VNTR probes (Jeffreys et al. 1985; Nakamura et al. 1987) offer reliable, feasible and rapid DNA analysis and are consequently efficient for resolving personal identification in many cases. But there are limiting factors for these VNTR analysis, for example in circumstances, where only small DNA amounts or partially degraded DNA are available. Because of the small sizes of STRs (≤ 350 bp), they can be informative in cases where VNTR analysis would fail.

STRs are a subset of microsatellite loci, containing a repeated motif of 2 to 7 nucleotids in length (Edwards, A. et al. 1991; Hammond et al. 1994; Weber et al. 1989; Alford et al. 1994). STRs differ in their number of nucleotids within their repeated sequence. The vast majority of STRs reported to date have been dinucleotide STRs, but increasing numbers of tri- and tetranucleotide STRs are being detected. The polymorphisms are due to the variable number of repeats of three, four and five nucleotide sequences. Three-, four-, and five-base repeats amplify more authentically and provide more easily interpretable results, than dinucleotide repeat loci do (Hammond et al. 1994; Edwards, M.C. et al. 1991; Huang et al. 1991). The tri- and tetranucleotide STRs have another significant advantage over dinucleotide STRs in that strand slippage artifacts (also called shadow or stutter bands), are greatly reduced (Dubovsky et al. 1995).

We have developed a PCR-based DNA typing method that uses 6 unlinked STR loci to provide a rapid and reliable typing system for personal identification with a highly discriminating power.

MATERIAL AND METHODS

Preparation of DNA

DNA was isolated from leucocytes, according to Miller et al. (1988).

Oligonucleotides

Oligonucleotides were synthesized with the DNA synthesizer „Gene Assembler Plus“ (Pharmacia, Freiburg, GER) and cleaned with NAP-10 columns (Pharmacia).

STR Loci

The STR loci used in this study are one trinucleotide STR locus (FABP2), four tetranucleotide STR loci (HPRT, F13A1, CYP19 and LPL) and one pentanucleotide STR loci (CD4).

Table 1 summarizes the STR locus names, Genome Database (GDB) designations, chromosomal locations, PCR primers and allele weight ranges of the six STR loci used in this study (Hammond et al. 1994).

Table 1: Characteristics of six unlinked STR-loci

Genbank Locus [STR] _n GDB Designation	Gene (chromosome location)	PCR Primers	Product Length (bp)
HUMHPRTB[AGAT] _n /HPRT	Hypoxanthine phosphoribosyltransferase (Xq26)	A:ATGCCACAGATAATACACATCCCC B:CTCTCCAGAATAGTTAGATGTAGG	259-299
HUMFABP[AAT] _n /FABP2	Intestinal fatty acid-binding protein (4q28-q31)	A:GTAGTATCAGTTTCATAGGGTCACC B:CAGTTCGTTTCATTGTCTGTCCG	199-220
HUMCD4[AAAAG] _n /CD4	Recognition/ surface antigen (cd4) (12p12-pter)	A:TTGGAGTTCGCAAGCTGAACTAGCG B:CCAGGAAGTTGAGGCTGCAGTGAA	125-175
HUMF13A01[AAAG] _n /F13A1	Coagulation factor XIII (6p24-p25)	A:GAGGTTGCACTCGAGCCCTTTGCAA B:TTCTGTAATCATCCCAGAGCCACA	281-331
HUMCYAR04[AAAT] _n /CYP19	Aromatase cytochrome P-450 (15q21.1)	A:GGTAAGCAGGTACTTAGTTAGCTAC B:GTTACAGTGAGCCAAGGTCGTGAG	173-201
HUMLIPOL[AAAT] _n /LPL	Lipoprotein lipase (8p22)	A:CTGACCAAGGATAGTGGGATATAG B:GGTAAC TGAGCGAGACTGTGTCT	125-175

PCR Conditions

DNA amount:

10-15 ng of genomic DNA.

Cycling conditions: (Gene Amp PCR System, 2400; Perkin Elmer thermocycler) 94°C, 5 min, 1 cycle; 94 °C, 30 s, then 60 °C, 45 s, then 72 °C, 30 s, 30 cycles; and 72 °C, 5 min, 1 cycle.

Reaction conditions:

Reagent:	Final concentration:
Buffer (100mM Tris-HCl, pH 8,3; 500mM KCl)	1 x
dNTPs	200 µM (of each)
Primers	0,25 µM (of each)
MgCl ₂	1,5 mM
Taq	1 unit/PCR

Electrophoresis and silverstaining

Amplified fragments were separated in a native polyacrylamide gel (7,5% T, 2% C), using a vertical electrophoresis system (17,1 x 32,5 x 0,08 cm, Model SA 32, GIBCO BRL). The amplified products of three different STR loci (HPRT-FABP2-CD4; F13A1-CYP19-LPL) were applied into one lane using a time distance of 15 minutes of intervening electrophoresis.

Settings: 500 V, 40 mA, 40 W, 2,5-3 hrs, detection by silverstaining (Bassam et al. 1991).

Allele designation

Exact allelic designation needs sequenced allelic ladders, which are still in preparation, according to the recommendations of Puers et al. (1994).

RESULTS AND DISCUSSION

Fig. 1 shows several DNA-profiles analyzed by PCR and vertical polyacrylamide gel electrophoresis, followed by silverstaining. All fragments are well separated and can be distinguished without any doubt. If the allelic ladders are finally developed, is it possible (i) to differentiate clearly alleles differing by only one repeat unit and (ii) to achieve a high output of efficient results. Together these findings suggest that these 6 STR loci are well suited for separation in one gel system.

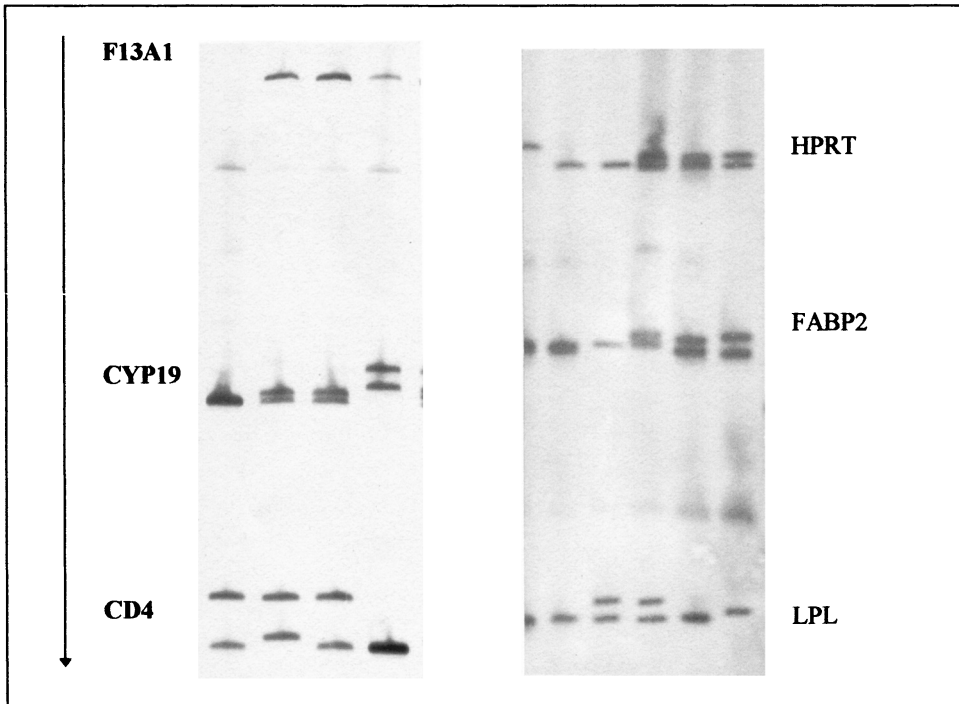


Figure1: Separation of six unlinked PCR-loci

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