MANUAL DNA TYPING VIA THE SHORT TANDEM REPEATS (STRs): KW 426, TH AND hTPO

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SUMMARY
Short tandem repeats (STRs) represent a rich class of highly polymorphic markers in the human genome. Usually they are composed of tandemly repeated sequences, 2 to 5 bp in length. Alleles range from 100 to 350 bp.
STRs have gained increasing popularity for genetic mapping and linkage analysis, trimeric and tetrameric loci are especially suited for identity testing.
We present a new combination of three unlinked tetrameric STR - loci (hTPO: 2p23 - 2pter, TH: 11p15.5, KW 426: 8q) performing the same polymerase chain reaction (PCR) - recipe for all three loci.
The amplification products of the three STR loci are separated together in one lane using a polyacrylamide-gradient-gel- electrophoresis (PAGGE) - system.
Thus we can type STRs with increased throughput and discrimination power by use of manual techniques which can easily be carried out by most laboratories.

1. INTRODUCTION

Short tandem repeats (STRs), also referred to as microsatellites or simple sequence length polymorphisms, occur approximately every 6 to 10 kb in the human genome (Olroyd 1995; Litt 1989; Tautz 1989).
Due to their abundance and high polymorphism they have become useful markers for physical and genetic mapping, personal identification and in some cases for disease diagnosis.
PCR fragment sizes are more and more often automatically determined by so-called "gene-scanners". Using these machines even octoplex STR typing is possible (Olroyd 1995).
Nevertheless many laboratories do manual DNA profiling because of the high costs of automatic systems. We have developed a procedure for:
1) the simultaneous PCR amplification and
2) the separation of three STR loci in one lane of a denaturing polyacrylamide-gradient-gel-electrophoresis (PAGGE) - system.

2. MATERIALS AND METHODS

2.1. DNA - PREPARATION:

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

2.2. OLIGONUCLEOTIDES

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

2.3. POLYMERASE CHAIN REACTION

Reaction conditions:
PCR-reaction conditions were: 1cycle 94°C/5min; 30 cycles 94°C/30 sec, 60°C/45 sec, 72°C/30 sec; 1 cycle 72°C/5 min.
Cycling conditions:
PCR was carried out in a 25 μl reaction mixture containing 200mM dNTPs, 0.5 U Taq DNA polymerase, 1 mM $MgCl_2$, 2.5 pmol of each primer for hTPO and 12.5 pmol of each primer for TH and KW 426 and 10 ng DNA.

Primers:
KW 426 (Lu 1993):
Primer 1: 5´- GTA GCC TCC CTG CCA TTT CCT AA -3´
Primer 2: 5´- TAT TGT GGT CCA GAG CTC CTT GG -3´
TH (Polymeropoulos 1991):
Primer 1: 5´- CAG CTG CCC TAG TCA GCA C -3´
Primer 2: 5´- GCT TCC GAG TGC AGG TCA CA -3´
hTPO (Anker 1992):
Primer 1: 5´- CAC TAG CAC CCA GAA CCG TC -3´
Primer 2: 5´- CCT TGT CAG CGT TTA TTT GCC -3´

2.4. Polyacrylamidegel electrophoresis and detection
PCR - fragments were separated in 6 - 10% denaturing polyacrylamide-gradient-gels at 600 V, 30 mA, 30 W for three hours.
Analysis was possible after silver staining.
Photographs were taken with the "Easy - Plus" - System (Herolab, Wiesloch, Germany).

2.5. Sample application
Samples were diluted 1 : 5 with water. 7.5 µl were then applied to the gel 2 cm from the cathode using paper sample sheets (0.5 x 0.5 cm; Pharmacia).

3. Results and Discussion
We describe a PCR procedure which enables simultaneous amplification of the three STR loci KW 426 (length: 322 - 382 bp), TH (244 - 260 bp) and hTPO (106 - 134 bp).
Table 1 summarizes characteristics of these loci.
Furthermore we demonstrate a new method of STR - PCR fragment analysis by using 6 - 10% denaturing polyacrylamide-gradient- gels (see figure 1).
Thus we present an easy reproduceable and cheap manual method to do STR DNA - profiling.

Exact allele typing demands an allelic ladder which is still in preparation. Large formal and population genetic studies will show whether this STR loci combination represents a highly informative genetic marker system.

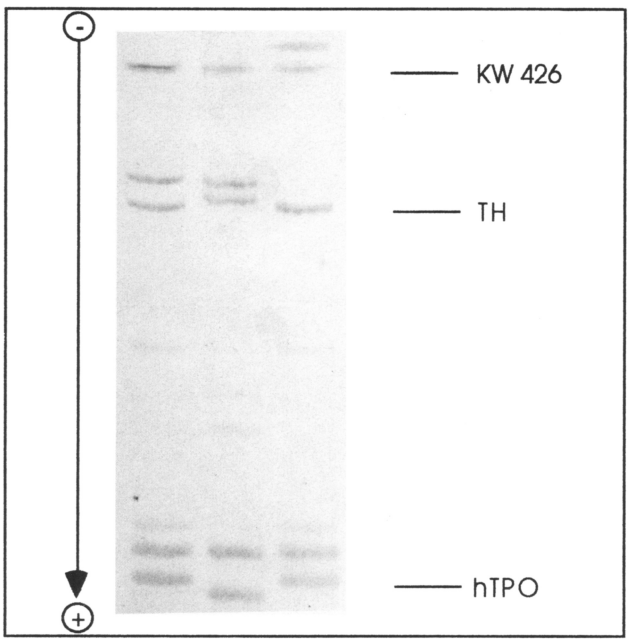

Fig.1: 6 - 10% PAGG showing STR separation; 600 V, 30 mA, 30 W for 3 hours

Table 1: Characteristics of the three STR - loci under investigation

STR:	description:	locus:	Genbank No:	alleles (bp):	repeat unit:	author:
KW 426	D8S347	8q	L12268	322-382	AGAT	Lu
TH	human tyrosine hydroxylase gene	11p15.5	D00269	244-260	TCAT	Polymer-opoulos
hTPO	human thyroid peroxidase gene	2p23-2pter	M68651	106-134	AATG	Anker

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