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SHORT COMMUNICATION

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Multiplex PCR amplification of TH01, D9S304, and D3S1744 loci

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Abstract A multiplex typing method of the tetrameric short tandem repeat (STR) loci TH01, D9S304, and D3S1744 was developed. The allelic ladder included alleles 6–11 (80–100 bp) and 9.3 (95 bp) for TH01, alleles 6–15 (125–161 bp) for D9S304, and alleles 13–22 (174–210 bp) for D3S1744. The observed heterozygosity of D9S304 was 0.851. The combined discrimination power of the three loci was 0.991.

Key words Forensic · DNA typing · STR · Multiplex PCR · TH01 · D9S304 · D3S1744

Introduction

Short tandem repeat (STR) loci consist of tandemly repeated sequences of two to five base pairs. Their abundance, hypervariability, and amenability to co-amplification by multiplex polymerase chain reaction (PCR) make them ideal DNA markers for use in individual identification (Edwards et al. 1991; Kimpton et al. 1994; Kimpton et al. 1996; Micka et al. 1996; Xiao et al. 1998; Lazaruk et al. 1998; Lins et al. 1998). Here, we describe a method for multiplex PCR of TH01 (Edwards et al. 1991), D9S304, and D3S1744 (Neuweiler et al. 1996; Perlee et al. 1996).

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Materials and methods

Three autosomal tetrameric STR loci (TH01, D9S304, and D3S1744) were used in this study. Their sequence data and their chromosomal locations were quoted from the Genome data base (Accession numbers TH01, D00269; D9S304, G08747; D3S1744, G08246).

Multiplex PCR was performed using 2–50 ng of genomic DNA in a 50-μl reaction volume. The reaction mix contained 15 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.16% bovine serum albumin (BSA), 0.1 U/μl Ampli Taq Gold (PE Biosystems, Foster City, CA, USA), and 0.25 μM each primer (Table 1). The thermal cycle was initial denaturing at 95°C for 11 min, followed by 26 cycles of denaturing at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 60°C for 60 min.

The allelic ladder was constructed, mixing the PCR products of the three loci. The allelic ladder included alleles 6–11 (80–100 bp) and 9.3 (95 bp) for TH01, alleles 6–15 (125–161 bp) for D9S304, and alleles 13–22 (174–210 bp) for D3S1744.

Results and discussion

We developed a multiplex typing system of TH01, D9S304, and D3S1744 loci for individual identification from evidential samples (Fig. 1).

The D9S304 locus was sequenced from seven homozygous samples and K562 DNA. The repeat unit of the D9S304 locus was GATA, and the sequences were identical to that deposited in the Genome Data Base.

The D3S1744 locus was sequenced from eight homozygous samples and K562 DNA. The basic repeat unit of the D3S1744 locus was GATA, as reported by Perlee et al. (1996). However, the number of TAGA flanked with GATA repeats was also variable. Five samples possessed $(TAGA)_2$, K562 DNA possessed $(TAGA)_3$, and the other

Table 1. Primer sequences of three short tandem repeat (STR) loci

THF	5′-CCT CCC TTA TTT CCC TCA T-3′
THR	5′-CTT CCG AGT GCA GGT CAC-3′
D9F	5′-CTC CAG TCT TTT TAA TAA TGA ATT-3′
D9R	5′-ATA TGT GCC CAC ACA CAT CT-3′
D3F	5′-GAC CAC TTC CAG TCC TCA CT-3′
D3R	5′-GCC ACT GCC TCT AAA ATT GT-3′

THR, D9R, and D3R primers were labeled with 5-FAM

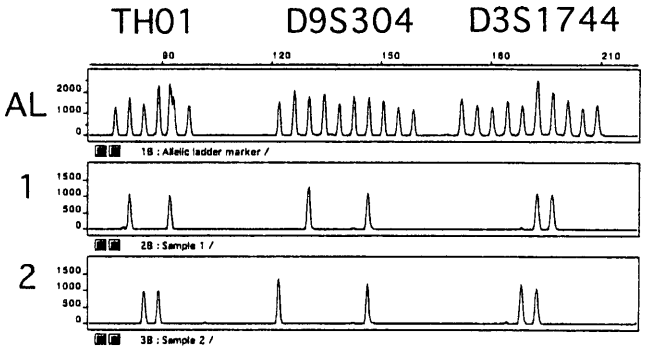

Fig. 1. Multiplex polymerase chain reaction amplification of TH01, D9S304, and D3S1744 loci. *AL*, Allelic ladder. *1*, Sample 1, alleles 7–9.3 (TH01), alleles 8–12 (D9S304), and alleles 18–19 (D3S1744); *2*, sample 2, alleles 8–9 (TH01), alleles 6–12 (D9S304), and alleles 17–18 (D3S1744)

three samples possessed KAKA followed by $(TAGA)_2$, where K represents G or T.

The distribution of allele frequency and the statistical properties of the D9S304 locus in a Japanese population are shown in Table 2. The observed heterozygosity of D9S304 (0.851) was slightly higher than that of TH01 (0.723 in Yoshida et al. 1996; 0.70 in Nagai et al. 1996) and that of D3S1744 (0.788 in Senju et al. 1999). The combined discrimination power of the three loci in a Japanese population was 0.991. This value reveals that this multiplex typing method is a powerful one for individual identification.

Table 2. Allele frequencies of D9S304 locus in a Japanese population

$n = 114$

D9S304 Allele	Frequency
6	0.066
7	0.018
8	0.355
9	0.053
10	0.079
11	0.075
12	0.167
13	0.118
14	0.061
15	0.009
Observed heterozygosity	0.851
Expected heterozygosity	0.809
Discrimination power	0.934
Polymorphism information content	0.789

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