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Typing of pentanucleotide STR polymorphisms

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Abstract

Pentanucleotide tandem repeat markers are of interest for forensic science because they may present less stutter in electrophoretic patterns. We focused on the analysis of the DNA sequence for each allele at two pentanucleotide STR loci, D6S957 and D10S2325, in order to understand their structures in the human genome and to construct allelic ladders, necessary for forensic DNA typing. In order to evaluate the forensic applicability of both pentanucleotide tandem repeat loci and to construct a preliminary database, the genotype distributions and allele frequencies in different ethnic groups were investigated. The population samples included Caucasians (Germans) and Asians (Chinese). The Amp-FLP technique was employed for DNA typing. An example of each allele and new alleles were sequenced. Allele determination for each pentanucleotide STR locus was carried out by comparison with a sequenced allelic ladder made in-house. Both pentanucleotide STR markers provided easily interpretable results. No evidence of deviation from Hardy–Weinberg equilibrium was observed. In 64 confirmed father/mother/child trios, no mutation event was observed. Using a maximum likelihood method, the mutation rate at both STR loci was indirectly estimated as 2.5×10^{-5}, suggesting both pentanucleotide STR markers would be useful for forensic casework and paternity analysis.

Keywords: Pentanucleotide tandem repeats; D10S2325; D6S957; DNA sequences; Polymorphism

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1. Introduction

Short tandem repeat loci have been shown to be very useful markers for forensic science [1]. Recently, many authors are developing pentanucleotide tandem repeat markers because they may present less stutter in electrophoretic patterns [2]. However, in order to be useful in forensic identity and paternity testing, any marker must display a significant degree of polymorphism within each major ethnic group. Based on this consideration, we evaluated the allele distribution in major ethnic groups for the pentanucleotide tandem repeat markers D10S2325 and D6S957. D10S2325 had previously been described as a forensic DNA marker by Lee et al. [3], while D6S957 was initially isolated as a sequence tagged site (STS) from the human genome and was labeled human STS UT1837 by the Utah marker development group [4].

2. Material and methods

2.1. Samples

Blood specimens were obtained from 100 Germans (Bremen, Germany) and 131 Chinese (Chengdu, Sichuan province, China), who were unrelated volunteers of blood bank donors. A total of 192 blood samples were collected from 64 confirmed father/mother/child triplets of Chinese living in Chengdu, China.

2.2. Experimental details

DNA was extracted using the Chelex method. PCR amplification was carried out using the primers according to the CHLC and Utah marker development groups [3,4].

D10S2325P1: 5′-CTCACGAAAGAAGCCTTCTG
D10S2325P2: 5′-GAGCTGAGAGATCACGCACT
D6S957P1: 5′-GGGCCGATGAAGTGATTGG
D6S957P2: 5′-TGGAGGGAGAGGGAAGTGC

Each PCR reaction contained 2–10 ng human genomic DNA, 1 × Taq buffer, 1.5 mM $MgCl_2$, 200 μM each nucleotide, 1.5 U Taq polymerase (Promega, USA), 0.25 μM each primer in a total volume of 37.5 μl. A total of 30 cycles were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer, USA) with denaturation for 50 s at 94 °C, annealing for 50 s at 59 °C and extension for 30 s at 72 °C. The PCR products were analyzed using nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system [5,6]. The allele classification was based on the number of repeat motifs [1].

2.3. Sequence analysis

PCR products were eluted from the gels and purified before sequencing. An example of each allele and new alleles were sequenced on an ABI 377 automated sequencer using a

Table 1
The DNA sequences of alleles at D10S2325 locus

Allele (bp)	P1(20 bp)aagcct(tctta)6–16ttgggggagacggactccgtcacccaggctggaatgc...P2(20 bp)
17 (168)	P1(20 bp)aagcct(tctta)17...37 bp...P2(20 bp)
19 (178)	P1(20 bp)aagcct(tctta)19...37 bp...P2(20 bp)
19a (178)	P1(20 bp)aagcca(tctta)19...37 bp...P2(20 bp)
21 (188)	P1(20 bp)aagcca(tctta)8(tcttg)7(tctta)2(tcttg)1(tctta)3...37 bp...P2(20 bp)

Dye Terminator Cycle Sequencing kit (PE Applied Biosystems, USA). The alleles were also cloned using the pGEM®-T Easy Vector System I (Promega) according to the manufacturer's instructions. The DNA clones were sequenced with the ABI 377 automated sequencer to verify the allele sequences.

3. Results and discussion

3.1. Sequencing data

Alleles at D10S2325 locus consisted of a simple pentanucleotide repeat structure except alleles 19 and 21. As shown in Table 1, there is a complex repeat structure with different motifs (tctta)*n* and (tcttg)*n* in allele 21, while at allele 19, a change of a single base in front of the repeat region was observed in different individuals. According to the analysis of sequence, allele 19 should be divided into two alleles, 19a and 19 (Table 1). There was a simple pentanucleotide repeat structure at D6S957 locus (Table 2).

3.2. Population genetics

Population data are shown in Table 3. No evidence of deviation from Hardy–Weinberg equilibrium in these populations was observed using the modified χ^2 test [7].

Table 2
The motifs of alleles at D6S957 locus

Allele (bp)	Sequence
9 (204)	P1(19 bp)...87 bp...(gcaca)9...34 bp...P2(19 bp)
10 (209)	P1(19 bp)...87 bp...(gcaca)10...34 bp...P2(19 bp)
11 (214)	P1(19 bp)...87 bp...(gcaca)11...34 bp...P2(19 bp)
12 (219)	P1(19 bp)...87 bp...(gcaca)12...34 bp...P2(19 bp)
13 (224)	P1(19 bp)...87 bp...(gcaca)13...34 bp...P2(19 bp)
14 (229)	P1(19 bp)...87 bp...(gcaca)14...34 bp...P2(19 bp)
15 (234)	P1(19 bp)...87 bp...(gcaca)15...34 bp...P2(19 bp)
16 (239)	P1(19 bp)...87 bp...(gcaca)16...34 bp...P2(19 bp)

Table 3
Allele frequencies and forensic value of D10S2325 and D6S957

D10S2325 allele	Germans Bremen	Chinese Chengdu	D6S957 allele	Germans Bremen	Chinese Chengdu
	Frequency	Frequency		Frequency	Frequency
6		0.008			
7	0.160	0.230			
8	0.045	0.031			
9	0.095	0.109	9		0.004
10	0.115	0.091	10		0.004
11	0.140	0.117	11		0.027
12	0.245	0.152	12	0.354	0.580
13	0.150	0.129	13	0.131	0.172
14	0.030	0.074	14	0.242	0.122
15	0.015	0.023	15	0.253	0.080
16	0.005	0.008	16	0.020	0.011
17		0.012			
19a		0.004			
19		0.004			
21		0.008			
Power of discrimination	0.953	0.966		0.883	0.802
Total alleles	200	256		198	262

3.3. Mutation rate

A total of 64 confirmed father/mother/child trios were analyzed. No mutation event was observed. Although the number of father/mother/child trios studied here is not very large, the mutation rates for D10S2325 and D6S957 seems to be reasonably low. Using data from these populations, the mutation rates for both loci were indirectly estimated with the maximum likelihood method of Chakraborty and Neel [8] to be 2.5×10^{-5}.

These results suggest that both pentanucleotide STR markers would make useful markers for forensic casework and paternity analysis.

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