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A new useful STR locus for forensic analysis

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Abstract

In order to get more available STR loci for forensic DNA analysis in Chinese populations, we investigated 10 tetranucleotide STR loci on chromosome 9 selected from GDB (D9S917, D9S254, D9S1121, D9S1118, D9S301, D9S1122, D9S922, D9S252, D9S2026, D9S934). Some of these were then chosen for further evaluation. 1039 EDTA-blood samples of unrelated individuals and some biological materials obtained from crime scene were used in this study. Amp-FLP techniques were employed for DNA typing. All alleles were cloned in plasmids and sequenced with an ABI 310. For each locus, the cloned alleles were amplified and the PCR products were mixed to make an allelic ladder for DNA typing. The software POPGENE was used to analyze the allele frequencies and heterozygosities of 10 STR loci in Chinese Han population. The results showed that compared with the other nine loci, allele frequencies of D9S1118 had a preferable distribution in Chinese Han population. The exclusion probability was 0.6405, while the discrimination power was 0.9424. Analyzing biological materials from crime scenes showed that typing D9S1118 had a higher success rate. All of these results suggest that D9S1118 as a marker could be used for forensic DNA analysis in Chinese populations.

Keywords: D9S1118; Chinese Han population; Polymorphism; STR

1. Introduction

In order to get more STR loci for forensic DNA analysis in the Chinese Han population, we selected 10 tetranucleotide STR loci from the CHLC bank. PCR amplification was employed to analyze allele frequencies and heterozygosity of 10 STR loci (D9S917,

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D9S254, D9S1121, D9S1118, D9S301, D9S1122, D9S922, D9S252, D9S2026, D9S934) [1]. Of all of them, D9S1118 has the most informative polymorphism in this population. To get more information about this locus, we focused on the analysis of the DNA sequence of each allele. The genotype distributions and allele frequencies in the Chinese Han population were also investigated.

2. Material and methods

2.1. Samples

EDTA-blood samples were collected from 1039 unrelated Chinese Han volunteers, who donated blood for blood banks in Chengdu, Sichuan province, China. Ethnic origin was determined by self-declaration. Additionally, EDTA-blood samples from parentage testing and some biological materials from the scenes of the crime, such as cigarette stubs, bloodstains, and remains, were also employed in this study.

2.2. Typing for D9S1118

Amplification primers for D9S1118 were from CHLC and were synthesized by us. The forward primer is 5′ -CAGGATATTATGTGATGGAATCC and the reverse primer is 5′ -CTGCTGACTCCAAAAATATGC. DNA was extracted by Chelex-100. All samples were amplified by polymerase chain reactions. Each PCR reaction contained 2–20 ng human genomic DNA, 1 U Taq buffer, 200 μM of each nucleotide (Pharmacia Biotech, Sweden), 1.5 U Taq polymerase (Roche, USA) and 0.25 μM of each primer in a total volume of 37.5 μl. In the PCR protocol, the DNA was initially denatured at 94 °C for 2 min. This was followed with 94 °C for 30 s, 56 °C for 1 min 15 s and 72 °C for 30 s. A total of 32 cycles were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer, USA). The PCR products of all samples were analyzed using a horizontal non-denaturing polyacrylamide gel electrophoresis with a discontinuous buffer system. The gel was silver stained.

2.3. Sequence analysis

Alleles were cloned using the pGEM®-T Easy Vector System I (Promega, USA) according to the manufacturer's instructions. To verify the allele sequences, positive clones were then selected and sequenced using an ABI 377 automated sequencer.

2.4. Nomenclature

The allele classification for D9S1118 was based on the number of repeat motifs according to the recommendations of the International Society of Forensic Haemogenetics (ISFH), [2].

2.5. Population genetics analysis

Genotyping of all samples was carried out by comparison with a sequenced allelic ladder, which was made by mixing the PCR products of the alleles cloned in this study.

Table 1
The DNA sequences of alleles at D9S1118 locus

Allele (bp)	P1 (23 bp) att (tatc) *n* aagatagggagaaagagagagaga gaagagatccttaagaataagctgtaggtaatattag P2 (21 bp)
8 (140)	P1 (23 bp) aat (tatc) 8. . .. . .61 bp. . .P2 (21 bp)
9 (144)	P1 (23 bp) aat (tatc) 9. . .. . .61 bp. . .P2 (21 bp)
10 (148)	P1 (23 bp) aat (tatc) 10. . .. . .61 bp. . .P2 (21 bp)
11 (152)	P1 (23 bp) aat (tatc) 11. . .. . .61 bp. . .P2 (21 bp)
12 (156)	P1 (23 bp) aat (tatc) 12. . .. . .61 bp. . .P2 (21 bp)
13 (160)	P1 (23 bp) aat (tatc) 13. . .. . .61 bp. . .P2 (21 bp)
14 (164)	P1 (23 bp) aat (tatc) 14. . .. . .61 bp. . .P2 (21 bp)
15 (168)	P1 (23 bp) aat (tatc) 15. . .. . .61 bp. . .P2 (21 bp)
16 (172)	P1 (23 bp) aat (tatc) 16. . .. . .61 bp. . .P2 (21 bp)
17 (176)	P1 (23 bp) aat (tatc) 17. . .. . .61 bp. . .P2 (21 bp)

The allele frequencies and the heterozygosity of this locus were calculated using the POPGENE software. Hardy–Weinberg equilibrium was also checked using the same software.

3. Results

3.1. Sequencing data

Analysis of DNA sequences showed that there was a simple repeat structure at the D9S1118 locus (Table 1).

3.2. Population data

There were 10 alleles at the D9S1118 locus in the Chinese Han population. Employing the POPGENE software, we analyzed the population genetic character of this locus. The results are shown in Tables 2 and 3.

Table 2
Genotype distribution in Chinese Han population ($n = 1039$)

Allele	8	9	10	11	12	13	14	15	16	17
8	36									
9	15	8								
10	4	3	5							
11	7	0	4	4						
12	34	10	6	7	25					
13	48	17	5	5	35	18				
14	28	13	1	6	17	19	12			
15	67	14	7	11	49	75	24	53		
16	44	10	1	3	36	46	25	59	32	
17	11	5	0	3	8	14	16	16	16	1

Table 3
Statistical description of D9S1118 ($n = 1039$)

Allele	Frequency	Statistical description	
8	0.1588	Hardy–Weinberg testing [3]	$\chi^2 = 43.884$
9	0.0496		$df = 44$
10	0.0197		$P > 0.05$
11	0.0260		
12	0.1218	Expected heterozygosity	0.8632
13	0.1449		
14	0.0833	Exclusion probability	0.6405
15	0.2060		
16	0.1463	Discrimination power	0.9424
17	0.0438		

3.3. Forensic genetics study

We amplified the DNA samples obtained from the scenes of the crime, such as cigarette stubs, bloodstains, and remains to test the amplified efficiency. The result showed that D9S1118 could give the same PCR products as the fresh EDTA-blood.

4. Discussion

According to our sequencing results, we named the alleles found in the D9S1118 locus from 8 to 17, with DNA sequences ranging from 140 to 176 bp. It should be mentioned that our alleles do not include allele 11.3, an allele that could be concluded from the GDB DNA sequence with 155 bp. The most likely explanation for the absence of allele 11.3 is the lower resolving power of the horizontal non-denaturing polyacrylamide gel electrophoresis, which was used to analyze the PCR products in this study. This electrophoresis system cannot distinguish the allele 11.3 and allele 12. The two alleles have a single base-pair difference. But for population studies there is no effect on our population data.

Our results show that the expected heterozygosity of D9S1118 is 0.8632. That means that this STR marker has a high level of polymorphism in the Chinese Han population. From the statistical analysis and the practical application in forensic genetics, we can conclude that D9S1118 could be used in the Chinese Han population.

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