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PowerPlex™ 16 analysis in the Japanese population

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Abstract

DNA typing was performed on 508 unrelated Japanese volunteers using the 15 short tandem repeats (STR) loci, that is, D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, and FGA, present in the PowerPlex™ 16 System. The allele frequencies, heterozygosity, power of discrimination, mean exclusion chance and polymorphism information content of all loci were calculated by statistical analysis. The combined matching probability and the combined mean exclusion chance in Japanese was 1 in 1.81×0^{17} and 0.9999989, respectively. None of the 15 loci were found to deviate from Hardy–Weinberg equilibrium (HWE) according to the results of the exact test and the homozygosity test. There was also no evidence for correlation of alleles between loci. This study demonstrates that this multiplex system is a useful and convenient tool for forensic identification and parentage testing in Japan.

Keywords: Short tandem repeat (STR); Japanese; Allele frequency; Multiplex PCR

1. Background

Short tandem repeats (STR) are widely used in forensics as well as in paternity testing. The introduction of multiplex PCR techniques has allowed simultaneous, rapid and robust amplification of several DNA loci from minute biological stains or fresh blood. In this study, we analyzed the 15 STR loci comprising the PowerPlex™ 16 System (Promega, Madison, USA) for allele frequency distributions and characteristics in the Japanese population. In addition, we verified the correlation among the loci.

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Table 1
Allele frequencies and statistical calculations for 15 loci in the Japanese population ($n=508$)

Allele	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	vWA	D8S179	TPOX	FGA	Allele
5					0.106											5
6		0.250														6
7		0.267				0.004	0.003			0.011	0.003					7
8		0.062			0.007	0.010	0.270	0.129	0.001		0.027			0.433		8
9		0.390			0.013	0.102	0.145	0.035	0.344	0.045	0.293		0.002	0.139		9
9.3		0.026														9.3
10		0.006		0.001	0.044	0.216	0.106	0.226	0.209	0.222	0.237		0.122	0.030		10
11				0.007	0.137	0.259	0.229	0.354	0.187	0.207	0.178		0.103	0.358		11
12	0.005			0.057	0.126	0.225	0.200	0.211	0.184	0.419	0.143		0.102	0.037		12
13				0.179	0.027	0.161	0.038	0.042	0.065	0.076	0.090	0.001	0.241	0.002		13
14	0.020			0.197	0.053	0.018	0.009	0.002	0.009	0.018	0.023	0.191	0.220	0.001		14
15	0.399			0.196	0.121	0.005			0.001	0.002	0.007	0.026	0.134			15
16	0.319			0.125	0.080							0.222	0.066			16
17	0.182			0.078	0.078							0.287	0.009		0.005	17
18	0.069			0.055	0.068							0.192			0.027	18
19	0.005			0.044	0.044							0.063			0.085	19
20	0.002			0.024	0.038							0.018			0.095	20
21				0.019	0.021										0.149	21
22				0.007	0.022										0.181	22
22.2															0.001	22.2
23				0.006	0.008										0.196	23
23.2															0.002	23.2
24				0.003	0.006										0.147	24
24.2															0.002	24.2
25				0.002	0.002										0.065	25

25.2															0.004	25.2
26				0.001											0.032	26
26.2																26.2
27															0.008	27
28			0.048												0.002	28
28.2			0.007													28.2
29			0.266													29
29.2			0.004													29.2
30			0.329													30
30.2			0.004													30.2
31			0.101													31
31.2			0.054													31.2
32			0.019													32
32.2			0.123													32.2
33			0.003													33
33.2			0.038													33.2
34																34
34.2			0.003													34.2
35																35
35.2			0.001													35.2
exact test	0.463	0.472	0.462	0.420	0.370	0.424	0.439	0.424	0.463	0.480	0.451	0.439	0.433	0.477	0.407	
homozygosity test	0.308	0.888	0.752	0.153	0.930	0.763	0.990	0.771	0.482	0.465	0.121	0.490	0.721	0.572	0.078	
H_{obs}	0.720	0.713	0.783	0.839	0.913	0.791	0.801	0.754	0.752	0.711	0.770	0.778	0.841	0.652	0.837	
H_{exp}	0.701	0.710	0.789	0.960	0.912	0.799	0.801	0.759	0.765	0.724	0.796	0.790	0.835	0.663	0.863	
PD	0.858	0.864	0.928	0.965	0.989	0.929	0.931	0.905	0.908	0.883	0.929	0.924	0.952	0.824	0.966	
PIC	0.648	0.658	0.761	0.844	0.907	0.768	0.772	0.722	0.728	0.683	0.767	0.757	0.814	0.601	0.847	
MEC	0.450	0.458	0.601	0.720	0.823	0.600	0.606	0.540	0.546	0.496	0.601	0.586	0.671	0.399	0.724	

2. Materials and methods

2.1. Sample preparation and PCR amplification

Buccal swab samples were obtained from 508 individual Japanese volunteers. DNA was extracted using Chelex100 method followed by phenol/chloroform extraction and ethanol precipitation.

We used the PowerPlex™ 16 System (Promega) to amplify 16 STR loci D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA and Amelogenin.

For PCR, 2 ng of genomic DNA was amplified in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, USA) according to the manufacturer's protocols.

2.2. Electrophoresis and data analysis

Electrophoresis was carried out on a 4% polyacrylamide sequencing gel on an ABI 377 Sequencer (Applied Biosystems) for 3 h using the GS34A-2400 run module. Fragment sizes were determined automatically using GeneScan™ 3.1.2 software (Applied Biosystems), and typed by comparison with the allelic ladder from the kit.

2.3. Statistical analysis

Allele frequencies in each locus were calculated and the exact test (the data were shuffled 10,000 times) [1] and the homozygosity test [2] were performed to evaluate Hardy–Weinberg equilibrium (HWE). The observed heterozygosity (H_{obs}), the expected heterozygosity (H_{exp}), the polymorphism information content (PIC), the mean exclusion chance (MEC) and the power of discrimination (PD) were also calculated. We determined the coefficients of correlation among various loci based on the theory of correlation function in multidimensional distribution [3].

3. Results and discussion

The observed allele frequencies for the 15 STR loci are shown in Table 1. The distribution pattern of 13 STR loci excluding the Penta E and Penta D loci were similar to our previous study [3]. The Penta E and Penta D loci, which were new pentanucleotide repeat markers, showed a high polymorphic parameter value for PIC, PD and MEC. In the D21S11, D18S51 and FGA loci, it is known there are many intermediate alleles comprising two nucleotide insertions or deletions in Caucasian, African and Hispanic-American populations. Although the minor alleles of D21S11 had a relatively high frequency, only nine alleles of FGA and no minor alleles of D18S51 were observed in this study. At the Penta E and TPOX loci, two 'allele 25' and one 'allele 14' were observed, respectively. They were larger than the allelic ladder markers accompanying the kit. The combined mean exclusion chance was 0.9999989 and the combined matching probability was 1 in 1.81×10^{17}. The genotype frequency distributions of all loci do not deviate from

Table 2
The coefficient of correlation among each locus (The average was −0.086 and standard error was 0.046 at each of all loci)

D3S1358	–														
TH01	0.045	–													
D21S11	0.015	0.085	–												
D18S51	−0.018	−0.085	−0.002	–											
Penta E	−0.020	0.017	−0.034	−0.105	–										
D5S818	0.081	−0.062	−0.015	−0.022	−0.010	–									
D13S317	0.045	0.002	0.013	−0.020	0.022	0.003	–								
D7S820	−0.079	−0.035	−0.006	0.095	−0.027	−0.084	0.022	–							
D16S539	0.002	0.086	0.064	−0.081	0.058	−0.054	−0.001	−0.085	–						
CSF1PO	−0.046	−0.003	0.008	−0.092	−0.021	0.026	0.010	0.033	−0.094	–					
Penta D	−0.009	−0.003	−0.066	0.078	−0.024	−0.099	−0.017	−0.029	0.057	0.006	–				
vWA	0.025	0.048	0.065	0.008	−0.019	0.029	0.031	0.027	0.011	−0.017	−0.061	–			
D8S179	−0.055	−0.021	0.016	−0.007	−0.110	0.036	−0.042	−0.039	−0.086	0.040	0.015	−0.028	–		
TPOX	−0.043	−0.088	0.022	0.045	0.006	−0.025	0.027	−0.028	0.046	−0.024	0.041	0.063	−0.056	–	
FGA	−0.036	−0.066	−0.015	0.026	0.025	0.051	−0.098	−0.037	−0.024	−0.004	−0.047	−0.011	0.016	−0.071	–
	D3S1358	TH01	D21S11	D18S51	Penta E	D5S818	D13S317	D7S820	D16S539	CSF1PO	Penta D	vWA	D8S179	TPOX	FGA

HWE expectations based on the exact test (the data were shuffled 10,000 times) and the homozygosity test.

The 11 loci in this system exist on different chromosomes. The D21S11 and Penta D loci, and the D5S818 and CSF1PO loci are located on chromosomes 21 and 5, respectively. Therefore, we examined the correlation between these two pairs of loci. The coefficients of correlation between the D21S11 and Penta D loci, and the D5S818 and CSF1PO, were −0.066 and 0.0261, respectively. No correlation was found between the two loci on the same chromosome, and they are statistically independent. In addition, Table 2 shows that there was no significant difference between each of all loci.

4. Conclusion

No correlation was found between any of the loci. The combined mean exclusion chance was 0.9999989 and the combined matching probability was 1 in 1.81×10^{17} in the Japanese population using the 15 STR loci studied [4].

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