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SHORT COMMUNICATION

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Allele frequencies of the six miniSTR loci in a population from Japan

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Abstract Allele frequencies and forensic parameters for the six miniSTR loci D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045 were investigated in a sample of 142 unrelated healthy Japanese individuals. The polymerase chain reaction (PCR) products contained within the six loci were less than 119 bp in size. The frequency distributions in the six short tandem repeat (STR) loci showed no deviations from Hardy–Weinberg equilibrium expectations. The accumulated powers of discrimination and power of exclusion for the six loci were 0.999998 and 0.98, respectively. It was thus considered that due to the small PCR products and the moderate degree of polymorphism, analysis with use of the six miniSTR loci was highly beneficial for the forensic analysis of degraded DNA.

Keywords MiniSTR · Japan · Population data

Introduction

The application of many short tandem repeat (STR) loci is regularly used in forensic practice for the clarification of personal identity [1, 2]. However, for highly degraded samples, STR loci amplification is practically impossible due to DNA fragmentation and the presence of polymerase chain reaction (PCR) inhibitors. In recent years, several successful methods for analyses of degraded samples by means of smaller-sized PCR products have been reported [3, 4]. Coble and Butler [5] conducted a literature-based research on 920 STR loci and reported new PCR primer designs for the STR loci D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045, which were not linked to the Combined DNA Index System (CODIS) markers. The PCR products for these six STR loci were less than 125 bp in size and proved to be highly beneficial in the testing of degraded DNA samples. Moreover, all loci showed a moderate degree of polymorphism in a US population sample involving 474 subjects. In this study, we investigated Japanese allele frequency distributions of the six short STR loci.

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Materials and methods

Blood samples were collected from 142 unrelated, healthy, adult Japanese individuals after informed consent was acquired. DNA was extracted from whole blood by using a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany).

Two triplex PCRs were performed with the primer sets designed by Coble and Butler [5]. PCR reactions were performed in a total volume of 10 μl containing 100 pg of genomic DNA, 1× GeneAmp PCR buffer (containing 1.5 mM $MgCl_2$), 200 μM of each deoxyribonucleotide triphosphate (dNTP, GeneAmp dNTP MIX), each primer set, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). One triplex PCR mixture contained 1.3 μM primer sets of D1S1677 (fluorescent dye labeled with NED), 0.7 μM D2S441 (VIC), and 1.1 μM D4S2364 (6FAM). The other triplex PCR contained 1.3 μM primers D10S1248 (6FAM), 1.3 μM D14S1434 (VIC), and 0.8 μM D22S1045 (NED). Amplification was carried out using GeneAmp 9700 (Applied Biosystems) in the 9600 mode. The PCR conditions used were modified from those of Coble and Butler [5]. Pre-PCR denaturation was performed at 95°C for 11 min followed by 28 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 45 s, and a final extension at 60°C for 30 min.

Electrophoresis was performed using an ABI 310 Genetic Analyzer (Applied Biosystems) and 1 μl of multiplex PCR product was mixed with 20 μl Hi-Di formamide and 0.5 μl of the GeneScan-500LIZ size standard. Samples

Table 1 Observed allele frequencies and forensic efficiency parameters for the six STR loci in a Japanese population

Allele	D1S1677	D2S441	D4S2364	D10S1248	D14S1434	D22S1045
8	–	–	0.028	–	–	0.190
9	0.007	–	0.197	–	–	–
10	0.011	0.229	0.479	0.004	–	–
11	0.011	0.363	0.296	0.004	–	0.021
11.3	–	0.018	–	–	–	–
12	0.130	0.222	–	–	–	0.327
13	0.433	0.032	–	0.123	–	0.218
14	0.338	0.127	–	0.296	0.151	0.201
15	0.063	0.011	–	0.243	0.165	0.035
16	0.007	–	–	0.218	0.053	0.007
17	–	–	–	0.092	0.211	–
18	–	–	–	0.018	0.366	–
19	–	–	–	0.004	0.046	–
20	–	–	–	–	0.007	–
OH	0.704	0.739	0.620	0.746	0.711	0.775
EH	0.677	0.746	0.643	0.782	0.767	0.767
P	0.977	0.63	0.299	0.741	0.902	0.958
MP	0.158	0.108	0.192	0.077	0.085	0.093
PD	0.842	0.892	0.808	0.923	0.915	0.907
PE	0.435	0.492	0.315	0.504	0.446	0.553
PIC	0.62	0.71	0.58	0.75	0.73	0.73
TPI	1.69	1.92	1.31	1.97	1.73	2.22

OH Observed heterozygosity, *EH* expected heterozygosity, *P* values of the exact tests for Hardy–Weinberg equilibrium, *MP* matching probability, *PD* power of discrimination, *PE* power of exclusion, *PIC* polymorphism information content, *TPI* typical paternity index

were injected for 2 s at 15,000 V and separated at 15,000 V at 60°C for 18 min. Data were analyzed using GeneScan 3.1.2 software. Allelic ladders for each allele typing were created according to Coble and Butler's protocol [5]. Moreover, at least two different homozygote samples in the observed size for each miniSTR locus were sequenced to calibrate repeat number.

The allele frequency at each locus was calculated from the numbers of alleles in the population samples. Hardy–Weinberg equilibrium was determined by an exact test using the GENEPOP (version 3.4) software package [6]. Forensic parameters were calculated using the PowerStats v1.2 software package (Promega, Madison, WI, USA, USA).

To compare this miniSTR strategy against commercial kit (Ampf*l*STR Identifiler, Applied Biosystems), highly degraded DNAs from nine tooth samples were tested. Each PCR reaction was performed in the same concentrations of template DNA. Analyses using Ampf*l*STR Identifiler kit were carried out according to the protocol in the user's manual.

Results and discussion

The observed allele frequencies and forensic statistical parameters for the loci in the Japanese population sample of 142 unrelated individuals are shown in Table 1. The genotype frequency distributions in the six STR loci showed no deviations from the Hardy–Weinberg equilibrium by an exact test. Heterozygosity values were greater than 0.7, except for D4S2364 (0.620). The power of discrimination ranged from 0.808 (D4S2346) to 0.923 (D10S1248), whereas the power of exclusion ranged from 0.315 (D4S2346) to 0.553 (D22S1045). The expected heterozygosity, power of discrimination, polymorphism information content, and typical paternity index of D10S1248 were highest among the loci. The accumulated power of discrimination and

Table 2 The result of allele typing using the miniSTR multiplex and Ampf*l*STR Identifiler kit

Sample no.	MiniSTR						Ampf*l*STR Identifiler
	D1S1677	D2S441	D4S2364	D10S1248	D14S1434	D22S1045	
1	+	+	+	+	+	+	3[a]
2	−	−	+	+	+	+	1
3	+	+	+	+	+	+	2
4	−	+	+	−	+	+	2
5	+	+	+	+	+	+	2
6	−	−	+	+	+	+	1
7	−	−	−	+	+	+	1
8	−	−	−	+	−	+	0
9	+	+	+	−	+	+	3

\+ Successful in typing, − unsuccessful in typing
[a]Number of loci detected except for amelogenin locus

power of exclusion for the six loci were 0.999998 and 0.98, respectively.

In the Japanese population, the PCR products for the STR loci are less than 119 bp in size, and all loci showed a moderate degree of polymorphism. In a previous paper, it was confirmed that these new locus assays were more successful for typing degraded bone samples than those of a commercial STR kit [5]. We also compared this miniSTR strategy against a commercial STR kit (Ampf*l*STR Identifiler, Applied Biosystems). Table 2 shows the number of loci successful in typing. The number of loci detected, except for amelogenin locus, in the Ampf*l*STR Identifiler kit ranged from 0 (sample 8) to 3 (sample 1), whereas in the miniSTR the range was from 2 (sample 8) to 6 (samples 1, 3, and 5). These results showed that the miniSTR strategy was more successful for typing degraded samples than those of a commercial STR kit. Moreover, we previously designed new PCR primers for four STR loci linked with the CODIS markers and reported that the analysis of the PCR products was more efficient for typing degraded DNA samples than those of a commercial STR kit [4]. Consequently, it was concluded that a combination of analyses of the miniSTR loci, in addition to assays using commercially available STR kits, is highly beneficial in the context of Japanese forensics practice involving personal identification from degraded DNA samples.

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