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Population study of small-sized short tandem repeat in Japan and its application to analysis of degraded samples

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Abstract. This paper reports on genetic population data of six small-sized short tandem repeat (STR) markers D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045 in samples from 100 unrelated Japanese individuals. In addition, testing was performed on highly degraded DNA to compare the analyses of the miniSTR loci against analyses performed using a commercial kit. Consequently, all loci showed a moderate degree of polymorphism and analyses using the loci were noted to be highly effective with the degraded DNA.

Keywords: miniSTR; Japan; Population data; Degraded DNA

1. Introduction

Short tandem repeat (STR) markers have been established as very useful tools in the clarification of personal identity. In the forensic practice of molecular post-mortem identification, we must sometimes analyze a variety of highly degraded DNA. One of the most effective methods of molecular analysis for highly degraded DNA is to reduce the size of the PCR products. Coble and Butler earlier reported on two triplex systems with short amplicon lengths for six autosomal STR loci (D1S1677, D2S441, D4S2364, D10S1248, D14S1434, and D22S1045) in the analysis of highly degraded DNA [1]. In this study, we present an analysis of Japanese population data with reference to the six miniSTR loci, and compare this system against a commercial STR kit using highly degraded DNA.

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2. Materials and methods

Fresh blood samples were collected from 100 unrelated, healthy, adult Japanese individuals after informed consent was obtained. DNA was extracted from whole blood using an SDS-Proteinase K treatment followed by the phenol/chloroform method. PCRs were carried out with the primer sets designed by Coble and Butler. PCR reactions were also performed according to their protocol, except for the PCR conditions established by GeneAmp 9700 (Applied Biosystems): pre-denaturation 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 data were analyzed using GeneMapper ID v3.2 software. Allelic ladders were formed using a combination of products of different lengths in each locus. At least two different homozygous samples in each locus were sequenced to calibrate the number of repeats. PCR was performed separately using each of the non-labeled primer sets reported by Coble and Butler. Hardy–Weinberg equilibrium was determined by an exact test using the GENEPOP (version 3.4) software package. To compare the miniSTR strategies against AmpflSTR Identifiler (Applied Biosystems), highly degraded DNA was used for testing. The DNA was extracted from three organs subject to decomposition and four bones using the phenol/chloroform method. The corresponding PCR reactions were performed in 30 cycles in the same concentrations of template DNA, and electrophoresis was performed under the same conditions.

3. Results and discussion

The observed amplicon lengths among 100 Japanese individuals ranged from 67 bp to 119 bp. The allele frequencies and *P*-values of the exact tests for Hardy–Weinberg equilibrium for each locus are shown in Fig. 1. None of the loci showed any deviation from Hardy–Weinberg equilibrium in the exact test, and all demonstrated a moderate degree of polymorphism. A comparison of allele typing

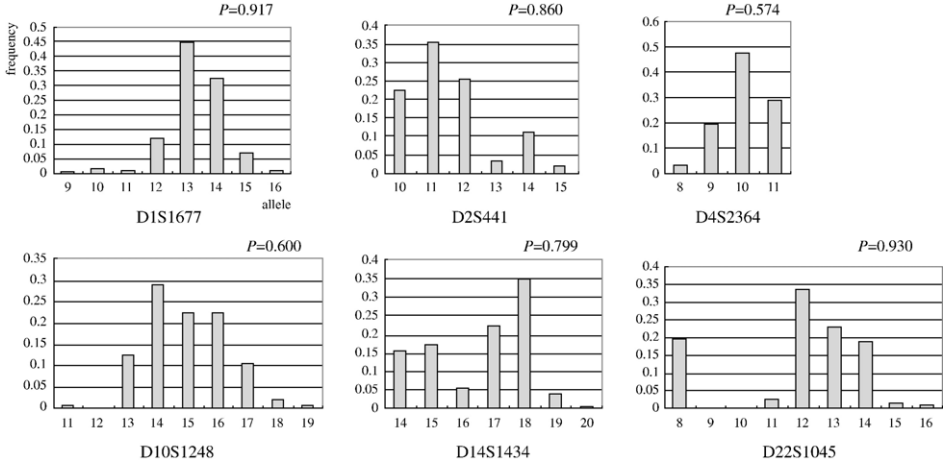

Fig. 1. Observed allele frequencies and *P*-values of the exact tests.

Table 1
The result of allele typing using the miniSTR multiplex and AmpflSTR Identifiler kit

Sample name	miniSTR loci						AmpflSTR Identifiler[a]	Total loci[b]
	D1S1677	D2S441	D4S2364	D10S1248	D14S1434	D22S10475		
DegDNA1	′+[c]	+	+	+	+	+	7	13
DegDNA2	+	+	+	+	+	′−[d]	3	8
DegDNA3	+	+	+	+	+	+	4	10
DegDNA4	−	+	−	+	+	+	3	7
DegDNA5	−	−	−	+	+	+	1	4
DegDNA6	−	−	−	+	−	+	0	2

[a] Number of loci detected except for amelogenin locus.
[b] Overall number of loci that were successful in typing with miniSTR strategy and AmpflSTR Identifiler kit.
[c] Successful in typing (+).
[d] Unsuccessful in typing (−).

for degraded DNA between these loci and the AmpflSTR Identifiler kit is shown in Table 1. These results show that the miniSTR strategy was more successful in typing degraded samples than the use of the AmpflSTR Identifiler kit. It was ultimately concluded that miniSTR loci analysis and use of the commercial kit form an effective combination in forensic practice.

Reference

[1] M.D. Coble, J.M. Butler, Characterization of new miniSTR loci to aid analysis of degraded DNA, J. Forensic Sci. 50 (2005) 43–53.