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Quadruplex amplification of polymorphic STR loci in a Korean population

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Abstract Multiplex PCR amplification has been useful for gene mapping with polymorphic short tandem repeat (STR) loci. We have tested the four loci D20S470, D13S325, HumFOLP23 and D10S2325 for the simultaneous typing of more than 100 unrelated Koreans. This analysis allows a single base pair resolution and rapid typing with silver staining. The allele and genotype distributions are in accordance with Hardy – Weinberg expectations. These STR loci have proven useful for forensic analysis and paternity tests in which the variable number of tandem repeat (VNTR) loci have some limitations.

Key words Quadruplex · Short tandem repeat · DNA typing · Korean population

Introduction

STR loci consist of tandemly repeated 2–7 bp sequences (Kimpton et al. 1993; Sprecher et al. 1996) and their great variability provide a rich source of polymorphic markers (Lee et al.1997). STR systems carrying tetranucleotide repeats produce fewer PCR artifacts than dinucleotide repeat loci (Weber and May 1989; Beckman and Weber 1992). Because of their small size (100–400 bp), STR systems are more useful for old or poorly stored specimens that contain degraded DNA. In order to establish our multiplex system, we carefully screened several STR loci having non-overlapping band patterns and compatible amplification conditions.

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Materials and methods

DNA preparation and amplification

Whole blood samples were obtained from the Red Cross National Blood Center. DNA preparation and amplification were performed using the method described by Park et al. (1997). The primer concentrations for each locus were 0.066 μM (D20S470), 0.132 μM (D13S325), 0.132 μM (HumFOLP23) and 0.264 μM (D10S2325).

Detection of amplified products

Discontinuous Tris-formate gel electrophoresis and silver staining method were performed as described by Budowle et al. (1991).

Genotyping

Allele designations, based on the number of repeat units, were determined by comparison with an allelic ladder (Bär et al. 1997). The nucleotide sequence of the repeat region of each amplified locus was determined by DNA sequencing.

Statistical calculations

Possible divergence from Hardy-Weinberg equilibrium was determined by using the χ^2-test (Evett et al. 1997). The polymorphism information content (PIC) was calculated using the method of Bostein et al. (1980). The power of discrimination (PD) was calculated using Fisher's equation (Fisher 1951).

Results and discussion

Quadruplex amplification of the polymorphic STR loci D20S470 (CHLC # 3201), D13S325 (Genbank # G09015), HumFOLP23 (Genbank # J00145) and D10S2325 (Genbank # G08790) were established for a Korean population sample and characteristics of the four loci are shown in Table 1. In setting up a multiplex system, a direct correlation between the primer dimer and product reduction was examined by Kimpton et al. (1996). Their results suggest that the primer dimer is related to enzyme activity and the amount of random primer/primer associations. In all samples, band intensities for each locus were found to be di-

Table 1 Characteristics of the STR loci used in this study

Locus	Chromosomal location	Repeat Sequences	Allele No*	Product length (bp)*	Primer sequence (5′→3′)
D20S470	20qter	TTCC	12	277–321	U:5′-CCTTGGGGGATATAGCCTAA-3′ L:5′-TGAGTGACAGAGTGATACCATG-3′
D13S325	13	AGAT	7	211–235	U:5′-TCCTTTAAGTGTCTAGAGAGGAGG-3′ L:5′-TCTCTCTCAGAAGTTTGGAAGC-3′
HumFOLP23	6	AAAC	6	162–182	U:5′-ATTGTAAGACTTTTGGAGCCATTT-3′ L:5′-TTCAGGGAGAATGAGATGGGC-3′
D10S2325	10	TCTTA	11	113–163	U:5′-CTCACGAAAGAAGCCTTCTG-3′ L:5′-GAGCTGAGAGATCACGCACT-3′

* our study in Korean population

Table 2 Allele frequencies (%) of the four STR loci in a sample of 102 Koreans

Allele[a]	Locus							
	D20S470 (n = 102)		D13S325 (n = 102)		HumFOLP23 (n = 102)		D10S2325 (n = 102)	
	frequency	length (bp)	frequency	length (bp)	frequency	length (bp)	frequency	length (bp)
4	0.00		0.00		0.00		0.00	
5	0.00		4.41	(211)	15.69	(162)	0.00	
6	0.00		21.57	(215)	44.61	(166)	2.45	(113)
7	0.00		34.31	(219)	13.73	(170)	25.98	(118)
8	0.00		20.59	(223)	4.90	(174)	2.94	(123)
9	3.43	(277)	12.75	(227)	6.37	(178)	13.24	(128)
10	11.76	(281)	4.90	(231)	14.71	(182)	10.29	(133)
11	3.43	(285)	1.47	(235)	0.00		9.80	(138)
12	2.45	(289)	0.00		0.00		10.78	(143)
13	8.82	(293)	0.00		0.00		13.73	(148)
14	20.59	(297)	0.00		0.00		6.86	(152)
15	12.75	(301)	0.00		0.00		2.94	(158)
16	19.12	(305)	0.00		0.00		0.98	(163)
17	8.33	(309)	0.00		0.00		0.00	
18	7.84	(313)	0.00		0.00		0.00	
19	0.98	(317)	0.00		0.00		0.00	
20	0.49	(321)	0.00		0.00		0.00	

[a] Allelic designation refers to the number of repeats of the core sequence motif indicated in the locus column
n, refers to the number of individuals sampled

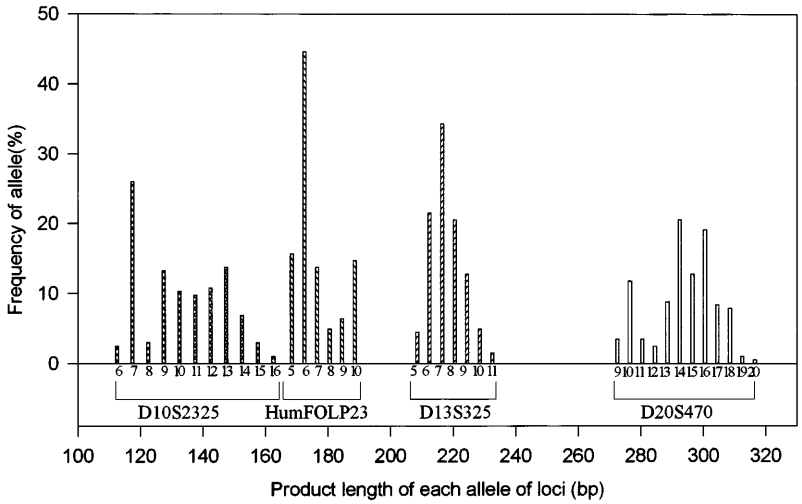

Fig. 1 Relative allele frequency histograms of the 4 STR loci studied. The data were generated from a minimum of 102 individuals from a Korean population. The distributions were plotted from the data (data not shown). Range of product length is represented on the X axis. Allele frequency (%) is shown on the Y axis. The numbers under the bars represent core sequence repeats of each loci

rectly related to primer concentration i.e the primer concentration for one locus seemed to affect the other primer concentration.

The distributions of observed allelic frequencies for the system used in this quadruplex are shown in Table 2. In particular, HumFOLP23 did not show any deviation

Table 3 Heterozygosity, *P*-value, PIC and PD values for the 4 loci under study

Locus	Observed Heterozygote %	*P*-value	PIC[a]	PD[b]
D20S470	87.25	0.253	0.8572	0.9614
D13S325	76.47	0.491	0.7477	0.9051
HumFOLP23	75.49	0.976	0.7051	0.8920
D10S2325	87.25	0.639	0.8549	0.9568

[a] PIC (polymorphism information content)
[b] PD (power of discrimination)

from expected values, which is consistent with the data of Park et al. (1997), even though only 100 samples were tested. This phenomenon was probably due to the historical and genetical homogeneity of the Korean population. Figure 1 shows frequencies and base lengths of products of the four loci in which the size range is about 110–320 bp. The observed heterozygosities for the loci D20S470, D13S325, HumFOLP23 and D10S2325 are 87.25%, 76.47%, 71.08% and 69.80% respectively. Table 3 shows the observed heterozygosity, PD, *P*-values and PIC values. No deviations from the Hardy-Weinberg equilibrium could be detected in these Korean samples. PIC values are also highly informative (PIC values are all > 0.5, Table 3) (Bostein et al. 1980).

In conclusion, this quadruplex system appears to be highly discriminating and results in reliable typing of four specific loci, thus providing a powerful tool for individual human identification and forensic applications.

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