Int J Legal Med (2006) 120:303–309
DOI 10.1007/s00414-006-0103-0

SHORT COMMUNICATION

Japanese population data for eight X-STR loci using two new quadruplex systems

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Received: 26 January 2006 / Accepted: 25 April 2006 / Published online: 7 June 2006
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Abstract Using two new X-chromosome short tandem repeats (X-STR) multiplex (quadruplex) systems, we investigated Japanese population data (drawn from 401 individuals) for eight X-STR loci (DXS10011, DXS9898, DXS8377, HPRTB, DXS7132, DXS6797, GATA172D05, and DXS6807). Allele typing with the systems was successful for all loci. The combined powers of discrimination of the eight loci in men and women were 0.999997 and 0.9999999996, respectively. We conclude that combined analyses of the eight X-STR loci using these two quadruplex polymerase chain reaction systems represent a powerful tool for Japanese forensic practice.

Keywords X-STR · Multiplex · Japanese population data

Introduction

Autosomal and gonosomal DNA polymorphisms are useful tools for determining personal identity [1, 2]. In particular, X-chromosome short tandem repeats (X-STR) analysis has been confirmed to be effective for kinship testing such as deficiency paternity cases. Population genetics data for numerous X-STR loci have recently been reported for certain ethnic groups, and X-STR multiplex polymerase chain reaction (PCR) systems, including a commercially available pentaplex kit (Mentype Argus X-UL, Biotype, Dresden, Germany), have also drawn attention [3–12]. In an earlier paper, we discussed mini X-STR multiplex systems for highly degraded DNA [13]. The systems successfully typed X-STR loci in DXS7423, DXS6789, DXS101, GATA31E08, DXS8378, DXS7133, DXS7424, and GATA165B12. In the study described in this paper, we investigated Japanese population data for eight other X-STR loci (DXS10011, DXS9898, DXS8377, HPRTB, DXS7132, DXS6797, GATA172D05, and DXS6807), using two new PCR quadruplex systems.

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00414-006-0103-0 and is accessible for authorized users.

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Materials and methods

Informed consent was obtained, and blood samples were drawn from 401 Japanese individuals (229 unrelated men and 172 unrelated women), corresponding 20 daughters and 14 sons (34 family trios). Parents of the children were included in the 401 individuals, while the paternity and maternity relationships of the families were confirmed by autosomal STRs genotyping using AmpflSTR Identifier Kit (Applied Biosystems, Foster City, CA, USA). DNA was extracted from whole blood using the Nucleic Acid Isolation System Quick Gene-800 (FUJIFILM, Tokyo, Japan).

Two multiplex PCRs were performed using the fluorescent dye-labeled primer sets shown in Table 1. PCR reactions were performed for a total volume of 10 μl consisting of 1 ng of genomic DNA, 1× GeneAmp PCR buffer, 1.5 mM of $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate (dNTP, GeneAmp dNTP MIX), each primer set, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems). The X-quadruplex-1 multiplex PCR mixture contained 0.8 μM primer sets of DXS10011 (fluorescent dye labeled with 6FAM), 1.2 μM DXS9898

Table 1 Primer sequences used in this study

X-STR locus	Primer sequences			
	Forward primer	Reverse primer[a]	Dye label	Reference
X-quadruplex-1				
DXS10011	5′-CTGAGATTGCACCATTGCAC-3′	5′-GTGGGAGAACCGTT TGAAGTT-3′	FAM	[14]
DXS9898	5′-CGAGCACACCTACAAAAGCT-3′	5′-GTCGATTAGGTTCAG TTCCCA-3′	VIC	[15]
DXS8377	5′-CACTTCATGGCTTACCACAG-3′	5′-GACCTTTGGAAAGC TAGTGT-3′	NED	[16]
HPRTB	5′-ATGCCACAGATAATACACATCCCC-3′	5′-GCTGTCTCTATTTCCA TCTCTGTCTCC-3′	PET	[17]
X-quadruplex-2				
DXS7132	5′-TCCCCTCTCATCTATCTGACTG-3′	5′-GTTTCTTTGCCAAACT CTATTAGTCAACG-3′	FAM	Newly designed[b]
DXS6797	5′-TTCCCTCTCTCCCTCTGTCT-3′	5′-GACACACACCCAAA ACCAGAT-3′	VIC	GDB[c]
GATA172D05	5′-TAGTGGTGATGGTTGCACAG-3′	5′-GATAATTGAAAGCC CGGATTC-3′	NED	[16]
DXS6807	5′-GAGCAATGATCTCATTTGCA-3′	5′-GAAGTAAACATGTATAGG AAAAAGCT-3′	PET	[18]

[a] A guanine was added to the 5′-end of each reference reverse primer to promote adenylation
[b] Newly designed primer set in this study
[c] Genome database (http://www.gdb.org)

(VIC), 0.8 μM DXS8377 (NED), and 0.8 μM HPRTB (PET). The X-quadruplex-2 multiplex PCR mixture contained 0.8 μM primer sets of DXS7132 (6FAM), 0.8 μM DXS6797 (VIC), 0.8 μM GATA172D05 (NED), and 1.2 μM DXS6807 (PET). The cycling programs were predenaturation at 95°C for 11 min, followed by 28 cycles of denaturing at 94°C for 30 s, annealing at 59°C for 30 s, extension at 72°C for 45 s, and a final extension at 60°C for 30 min with a GeneAmp 9700 (Applied Biosystems) in 9600 mode. Electrophoresis was performed with an ABI 3100-Avant Genetic Analyzer (Applied Biosystems), in which 0.5 μl of PCR product was mixed with 9.3 μl Hi–Di formamide and 0.2 μl of the GeneScan-500LIZ size standard. GeneMapper ID v3.2 software was used to analyze the data. Allelic typing was based on sequenced allelic ladders (supplementary Fig. 1 in Appendix). Genotypes for two commercially available cell lines K562 (Promega, Madison, WI, USA) and 9947A (Applied Biosystems) were typed using the allelic ladder. To determine the minimum quantity of DNA required to achieve reliable results (peak highs in all loci exceeded 200 relative fluorescence units) with the multiplex systems, we used a dilution series of 9947A DNA (50,100, 250, and 500 pg, and 1, 5, and 10 ng).

We calculated various forensic statistical parameters by the methods described in the earlier papers: polymorphism information content (PIC) [19], expected

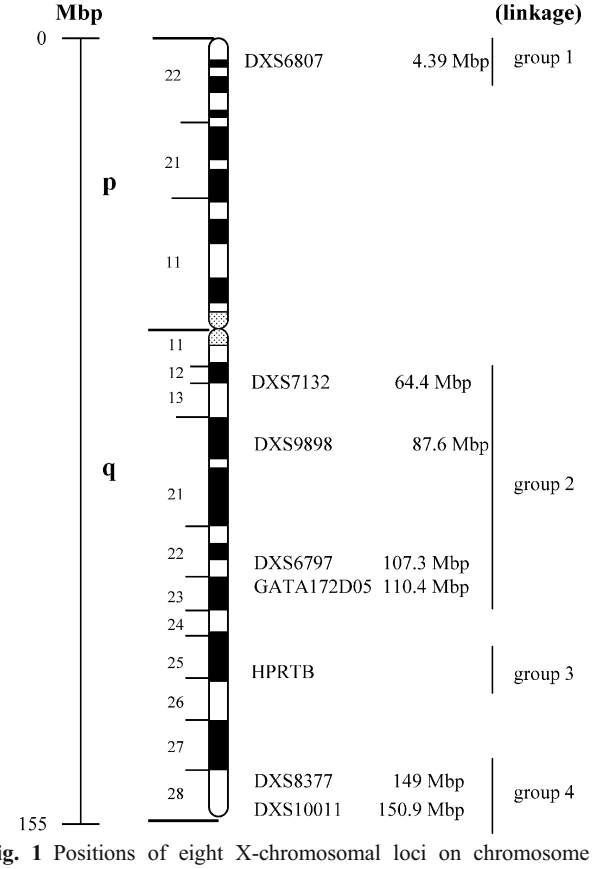

Fig. 1 Positions of eight X-chromosomal loci on chromosome X ideogram. Detailed positions were obtained in mega base pairs (http://www.ncbi.nlm.nih.gov). Linkage groups are according to Szibor et al. [21, 22]

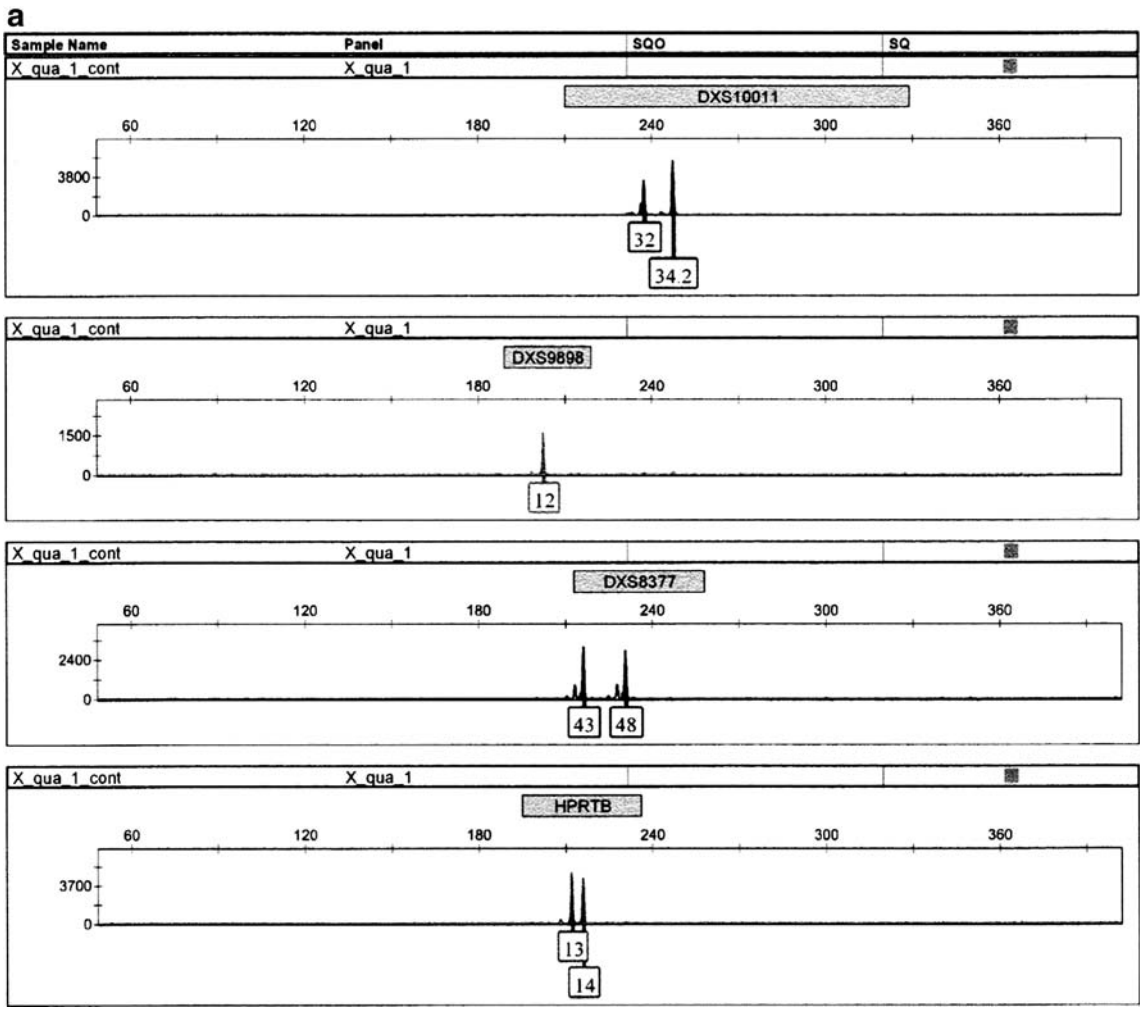

Fig. 2 Electropherograms of the two quadruplex systems under a woman sample. **a** X-quadruplex-1. **b** X-quadruplex-2

probability of exclusion (PE) [20], power of discrimination (PD) [20], and mean exclusion chance (MEC) [21].

Results and discussions

The eight X-STR loci were analyzed using the two new PCR quadruplex systems. These X-STR loci included representatives from each linkage group [21, 22], (group 1: DXS6807; group 2: DXS7132, DXS9898, DXS6797, and GATA172D05; group 3: HPRTB; and group 4: DXS8377 and DXS10011). Figure 1 gives the locations of the eight loci on the X-chromosome. DXS6797 locus contains two STRs separated by 128 nonpolymorphic nucleotides. Although haplotype analysis of both the STRs would increase the amount of information compared with that of the complex double STR [23], in this study, we investigated the length of the complex double STRs amplicon. PCR amplification and allele typing with the systems were successful for all eight loci (Fig. 2). Genotypes for two commercially available cell lines (K562 and 9947A) are given in Table 2. Stutter peaks were less than 10% except for the DXS8377 loci. Although the height of the stutter peaks ranged from 20 to 30% in DXS8377, in all women samples, each heterozygote peak always showed approximately the same height and false allele typing due to stutter peaks proved to be impossible. The minimum quantity of DNA required to obtain reliable results in the systems was determined to be 250 pg for X-quadruplex-1 and 100 pg for X-quadruplex-2. The observed allele frequencies and forensic statistical parameters for the eight X-STR loci were determined after an investigation of genetic data from 229 Japanese men and 172 Japanese women (Table 2). Heterozygosity values ranged from 0.552 (HPRTB) to 0.942 (DXS10011), PIC ranged from 0.597 (DXS6807) to 0.959 (DXS10011), while PE ranged from 0.441 (HPRTB) to 0.921 (DXS10011). The PD for men data ranged from 0.639 (DXS9898) to 0.953 (DXS10011), while the PD for women ranged from 0.818 (DXS6807) to 0.997 (DXS10011). The combined PDs for the eight loci in men and women were 0.999997 and 0.9999999996, respectively. The MECI ranged from 0.388 (DXS6807) to

Fig. 2 (continued)

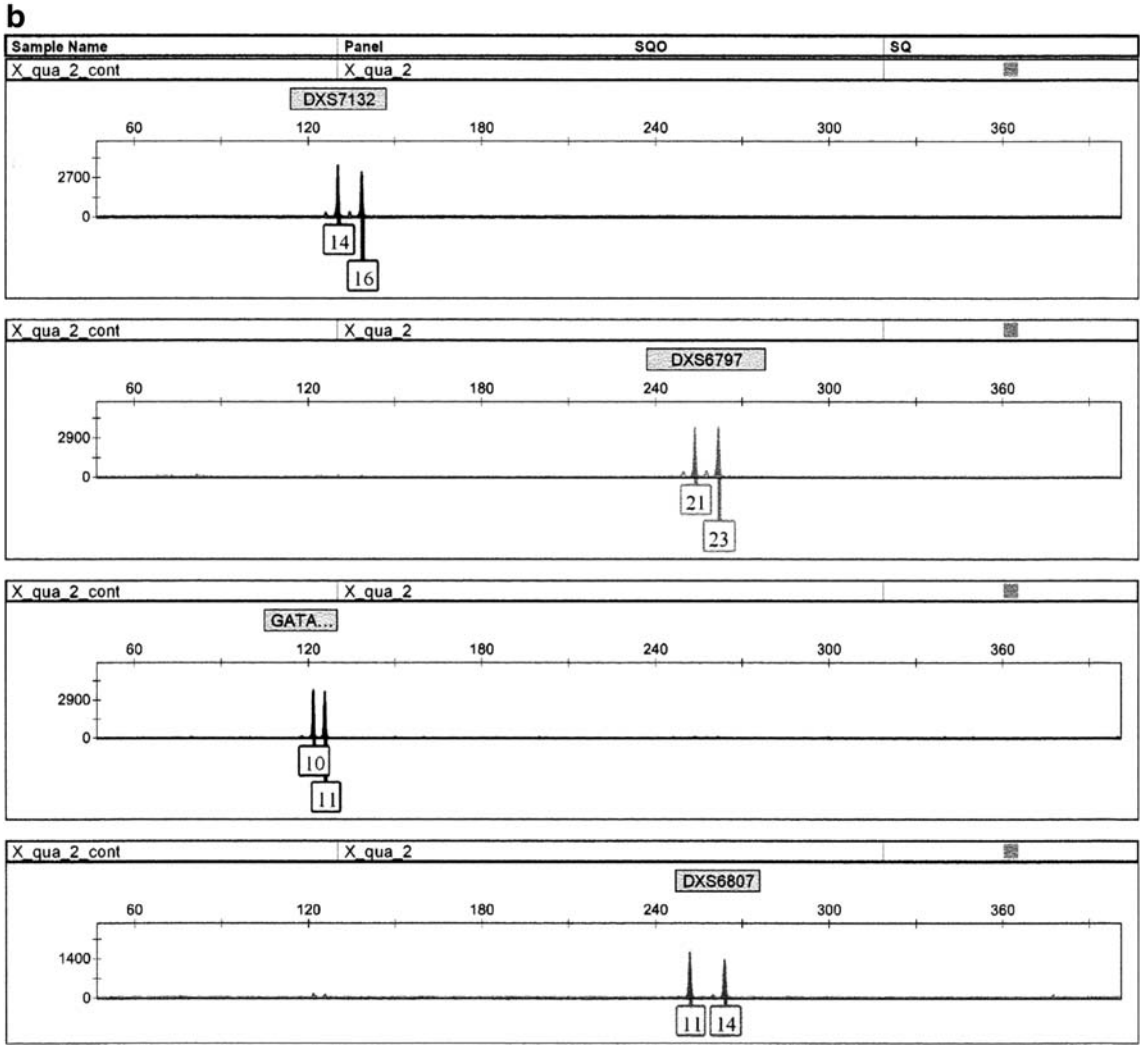

Table 2 Genotype of standard DNA and Japanese population data of eight X-STR loci

		X-quadruplex-1				X-quadruplex-2			
		DXS10011	DXS9898	DXS8377	HPRTB	DXS7132	DXS6797	GATA172D05	DXS6807
Cell-line DNA	K562	29	12	52	13	13	24	12	11
	9947A	36–38	12–15	45–47	14–14	12–12	21–23	10–10	12–14
Number of typed	Men	229	229	229	229	229	229	229	229
chromosomes	Women	172	172	172	172	172	172	172	172
Allele									
6								0.096	
7									
8								0.120	
8.3			0.042						
9								0.059	
10			0.005					0.442	
11			0.085		0.033	0.002		0.229	0.363
12			0.508		0.269	0.059		0.054	0.005

Table 2 (continued)

	X-quadruplex-1				X-quadruplex-2			
	DXS10011	DXS9898	DXS8377	HPRTB	DXS7132	DXS6797	GATA172D05	DXS6807
13		0.260		0.490	0.199			0.021
14		0.065		0.140	0.326			0.412
15		0.033		0.056	0.300			0.187
16		0.002		0.012	0.101			0.012
17					0.011	0.002		
18					0.002	0.011		
19						0.005		
20						0.011		
21						0.253		
22						0.375		
23						0.249		
24						0.059		
25	0.002					0.031		
26	0.003					0.002		
27	0.010					0.002		
28	0.021							
28.2	0.002							
29	0.021							
30	0.037							
30.2	0.030							
31	0.044							
31.2	0.037							
32	0.056							
32.2	0.052							
33	0.033							
33.2	0.060							
34	0.038							
34.2	0.040							
35	0.040							
35.2	0.016							
36	0.056							
36.2	0.007							
37	0.051							
38	0.066							
39	0.045							
40	0.042							
41	0.045							
42	0.038		0.012					
43	0.026		0.028					
44	0.024		0.024					
45	0.021		0.070					
46	0.009		0.100					
47	0.009		0.129					
48	0.005		0.147					
49	0.005		0.171					
50	0.003		0.105					
51	0.002		0.063					
52	0.002		0.047					
53			0.030					
54			0.033					
55	0.002		0.026					
56			0.005					
57			0.007					
58			0.003					
HET[a]	0.942	0.640	0.901	0.552	0.587	0.669	0.674	0.657

Table 2 (continued)

	X-quadruplex-1				X-quadruplex-2			
	DXS10011	DXS9898	DXS8377	HPRTB	DXS7132	DXS6797	GATA172D05	DXS6807
PIC[b]	0.959	0.615	0.889	0.614	0.709	0.683	0.686	0.597
PE[c]	0.921	0.484	0.812	0.441	0.561	0.537	0.531	0.447
PD (men)[d]	0.953	0.639	0.887	0.694	0.757	0.731	0.736	0.663
PD (women)[e]	0.997	0.850	0.982	0.819	0.891	0.878	0.878	0.818
MECI[f]	0.916	0.428	0.791	0.420	0.523	0.491	0.506	0.388
MECII[g]	0.957	0.616	0.887	0.614	0.709	0.682	0.686	0.596

[a]Observed heterozygosity
[b]Polymorphism information content [19]
[c]Power of exclusion [20]
[d]Power of discrimination in men [20]
[e]Power of discrimination in women [20]
[f]Mean exclusion chance for autosomal markers in trios [21]
[g]Mean exclusion chance for X-chromosome markers in trios [21]

0.916 (DXS10011), while the MECII ranged from 0.596 (DXS6807) to 0.957 (DXS10011). A comparison of these allele frequencies based on data for Japanese and Korean [8] showed no significant differences. On the investigated kinship cases (34 family trios), no mutation was detected, although the number of meioses investigated was too small to estimate a reliable mutation rate. The combined analyses of the eight X-STR loci using the two quadruplex PCR systems discussed in this study would appear to present a powerful tool for Japanese forensic practice.

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