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ORIGINAL ARTICLE

Sixteen X-chromosomal STRs in two octaplex PCRs in Japanese population and development of 15-locus multiplex PCR system

Yasutaka Nakamura · Kiyoshi Minaguchi

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Abstract X-chromosome short tandem repeat (STR) polymorphisms are a useful tool in the fields of human population genetics and personal identification and are indispensable in investigating complex kinship or deficiency cases in circumstances where information on mtDNA or Y-chromosome polymorphisms is unavailable. The purpose of this study was to construct a multiplex polymerase chain reaction (PCR) system capable of analyzing a large number of X-STR loci and establish a 16-X-STR database in the Japanese population We developed two octaplex X-STR systems, one including the DXS7424, GATA172D05, HPRTB, DXS8377, GATA31E08, DXS9895, DXS7423, and DXS981 loci and the other the DXS6803, DXS6789, DXS6800, DXS6809, DXS7133, DXS7132, DXS101, and DXS6807 loci, and conducted a population study in 512 Japanese individuals comprising 339 men and 173 women. A 16-locus multiplex system produced unwanted PCR products due to mixture of the DXS9895 primer with the primers of two other loci. However, a 15-locus multiplex system exclusive of the DXS9895 locus did not. The 15-locus multiplex system amplified the largest number of loci among the X-STR multiplex systems used and afforded a power of discrimination of 0.99999999999997 in women and 0.999999997 in men.

Keywords X-Chromosome · STR · Japanese · Octaplex PCR · 15-Locus multiplex PCR

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Y. Nakamura · K. Minaguchi (✉)
Department of Forensic Odontology, Tokyo Dental College,
1-2-2 Masago, Mihama-ku,
Chiba City 261-0011, Japan
e-mail: minaguci@tdc.ac.jp

Introduction

The human X chromosome has been the focus of much research in the fields of population genetics and forensics in recent years, e.g., [1–14]. X-chromosomal short tandem repeats (X-STRs) can be used to complement autosomal STRs in paternity testing of female children or maternity testing of male children. They are considerably effective, especially in cases where Y-chromosomal or mitochondrial DNA polymorphisms are of no use and only X-chromosomal haplotypes are available for confirmation of blood relationships. For forensic application, however, it is important to collect population data and construct reference databases documenting genetic variation in specific STRs within a given population. Furthermore, an investigator is sometimes faced with only a small amount of DNA to work with or has to determine inheritance of an X-STR haplotype from many individuals. In such cases, it is necessary to keep use of samples or time required for running procedures down to a minimum.

The aim of this study was to construct a multiplex polymerase chain reaction (PCR) system capable of analyzing a large number of X-STR loci and obtain the allelic frequencies of many X-STR loci in a Japanese population. We believe that these data and the present multiplex system will prove useful in future human population genetic and forensic studies.

Materials and methods

Samples

Genomic DNA was extracted from blood samples obtained from 512 unrelated Japanese individuals (339 men and 173

women). Informed consent was obtained from all donors. This study was approved by the Ethics Committee of Tokyo Dental College. Leukocyte preparations from the blood samples were digested with proteinase K (Sigma-Aldrich) at 55°C overnight, followed by treatment with RNAse at 55°C for 2 h. DNA was extracted with phenol/chloroform, precipitated with ethanol and resuspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA at pH 7.6).

PCR amplification and typing of X-STRs

Sixteen X-STR loci (DXS7424, GATA172D05, HPRTB, DXS8377, GATA31E08, DXS9895, DXS7423, DXS981, DXS6803, DXS6789, DXS6800, DXS6809, DXS7133, DXS7132, DXS101, and DXS6807) were examined for polymorphisms (Fig. 1). These loci were divided into four groups (groups 1 to 4) according to size of amplified product (Table 1). Group 1, containing DXS7424, GATA172D05, HPRTB, and DXS8377, was labeled with 6-FAM; group 2, containing GATA31E08, DXS9895, DXS7423, and DXS981, was labeled with VIC; group 3, containing DXS6803, DXS6789, DXS6800, and DXS6809, was labeled with NED; and group 4, containing DXS7133, DXS7132, DXS101, and DXS6807, was labeled with PET. Multiplex PCR was performed in two single PCR reactions, each amplifying the combination of two different groups of X-STRs: the combination of groups 1 and 2 (group 1+2), or the combination of groups 3 and 4 (group 3+4). The primer sequences, concentrations used in the multiplex, type of labeled dye and range of amplified fragment sizes are listed in Table 1. New primers were designed for DXS981 and DXS6789 to adjust fragment length in the octaplex PCR. A new primer was also designed for DXS6800 to avoid possible amplification of an extra band in the 15-locus multiplex system (see Results and discussion sections for further explanation on this point). However, the sequences of the other primers were obtained from previous monographs [1, 7, 15–18]. Multiplex PCR was performed in a volume of 25-μl reaction mix containing: 1~10 ng genomic DNA, 10 mM Tris–HCl at pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 200 μM dNTP, 1.5 U AmpliTaq Gold (Applied Biosystems), and an appropriate volume of each primer (Table 1). The PCR temperature profile for the groups 1+2 multiplex was as follows: 11 min at 95°C followed by 50 s at 95°C and 105 s at 60°C for 28 cycles, with a final extension at 60°C for 30 min. The PCR temperature profile for the groups 3+4 multiplex was as follows: 11 min at 95°C followed by 50 s at 95°C and 105 s at 58°C for 28 cycles, with a final extension at 60°C for 30 min. Twelve microliters Hi-Di formamide (Applied Biosystems) and 0.5 μl GeneScan-500 LIZ internal size standard were added to each PCR product. Electrophoresis was performed using the ABI PRIZM 310 Genetic Analyzer (Applied Biosystems). Fragment sizes were automatically determined using the GeneScan Analysis software 3.1 (Applied Biosystems), and results were analyzed using the Genotyper ver. 2.5 (Applied Biosystems). Genotyping was performed by comparing the sequenced samples with the DNA control reference sample 9947A (Applied Biosystems) to validate the typing protocol for multiplex X-chromosomal STRs [19].

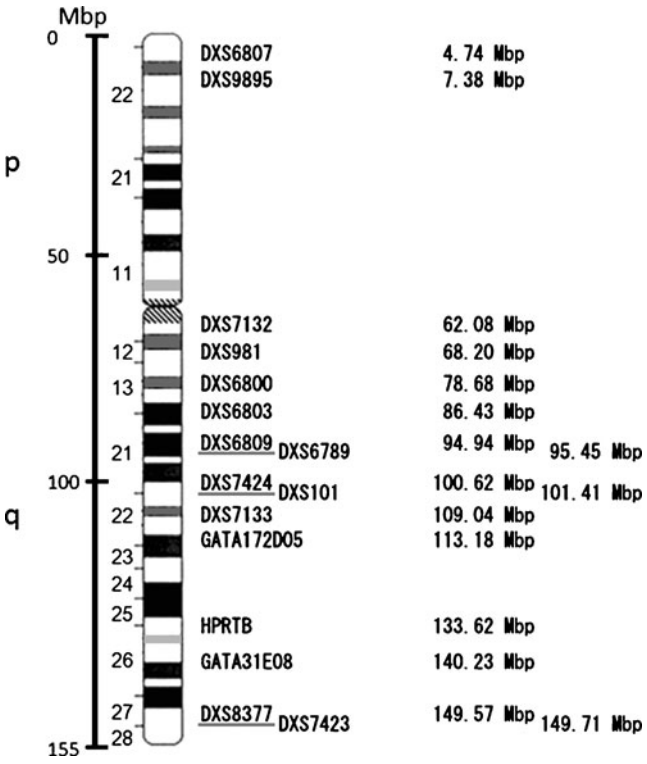

Fig. 1 Location of 16 STR loci studied on X-chromosome. Physical localization is given in megabase pairs

Sequencing analysis

Before employing our dye-labeled multiplex system, we conducted non-labeled multiplex PCR by a method similar to the one described above and compared many samples by electrophoresis in 6% denaturing polyacrylamide gel followed by silver staining. For a comparison with the established allele nomenclature of the targeted X-STRs (http://www.chrx-str.org), several allelic products from all X-STR loci were eluted from the gel, re-amplified by PCR, and directly sequenced, or PCR products from hemizygous male participants were directly sequenced. Amplicons were purified with the PureLink PCR purification kit (Invitrogen) according to the manufacturer's instructions. PCR for sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Excessive dye was removed using Performa DTD Gel Filtration Cartridges (EdgeBio) or the BigDye XTerminator Purification Kit (Applied Biosystems).

Table 1 Primer sequences in this study

Locus	Size (bp)	Primer sequence		Dye labeled	Reference	Final primer concentration (pmol)
Group 1						
DXS7424	76–100	F:AAACACAGGAAGACCCCATC	R:GGCTAAGAAGAATCCCGCACA	6-FAM	[7]	0.09
GATA172D05	108–132	F:TAGTGGTGATGGTTGCACAG	R:ATAATTGAAAGCCCGGATTC	6-FAM	[1]	0.38
HPRTB	144–176	F:TCTCTATTTCCATCTCTGTCTCC	R:TCACCCCTGTCTATGGTCTCG	6-FAM	[1]	0.95
DXS8377	201–261	F:CACTTCATGGCTTACCACAG	R:GACCTTTGGAAAGCTAGTGT	6-FAM	[15]	0.57
Group 2						
GATA31E08	101–129	F:CAGAGCTGGTGATGATAGATGA	R:GCTCACTTTTATGTGTGTATGTATCTCC	VIC	[7]	0.08
DXS9895	139–163	F:TTGGGTGGGGACACAGAG	R:CCTGGCTCAAGGAATTACAA	VIC	[15]	1.22
DXS7423	179–191	F:GTCTTCCTGTCATCTCCCAAC	R:TAGCTTAGCGCCTGGCACATA	VIC	[15]	0.12
DXS981	215–247	F:GTTTCCTCCTGCAAAATACAGC	R:TCCAGCACCCAAGGAAGTC	VIC	This study	0.08
Group 3						
DXS6803	105–128	F:GAAATGTGCTTTGACAGGAA	R:CAAAAAGGGACATATGCTACTT	NED	[16]	1.66
DXS6789	134–178	F:TTGGTACTTAATAAACCCTCTTT	R:GTCCTATTGTATTAGTCAGG	NED	This study	1.66
DXS6800	190–218	F:GTGTGAGTTTAATACTCCTTAAT	R:CTCTTTATTTCTCAGACTGGC	NED	This study	1.66
DXS6809	231–278	F:TGAACCTTCCTAGCTCAGGA	R:TCTGGAGAATCCAATTTTGC	NED	[17]	3.33
Group 4						
DXS7133	80–100	F:AGCTTCCTTAGATGGCATTCA	R:GTTTTTAACGGTGTTCATGCTT	PET	[7]	0.9
DXS7132	131–155	F:GAGCCCATTTTCATAATAAA	R:GCCAAACTCTATTAGTCAAC	PET	[15]	5.4
DXS101	197–230	F:ACTCTAAATCAGTCCAAATATCT	R:AAATCACTCCATGGCACATGTAT	PET	[18]	3.6
DXS6807	251–275	F:GAGCAATGATCTCATTTGCA	R:AAGTAAACATGTATAGGAAAAAGCT	PET	[1]	3.6

Sequence analysis was performed on an ABI PRISM 3100 automated sequencer (Applied Biosystems).

Statistical analysis

The chromosomal location of the 16 markers was determined by querying the NCBI map viewer. Observed heterozygosity (Hobs) was calculated using female data with the PowerStatsV12 software (http://www.promega.com). Polymorphism information content, power of discrimination in female, power of discrimination in males, and power of exclusion were also calculated with the PowerStatsV12 software. Linkage disequilibrium and Hardy–Weinberg equilibrium were determined with an exact test using the GENEPOP software (ver. 3.4; http://genepop.curtin.edu.au).

Results and discussion

Construction of two octaplex PCR systems and allele designation

We selected 16 X-STR loci distributed over the entire human X chromosome (Fig. 1). These loci were selected as they had previously been examined in other populations (http://www.chrx-str.org), and their data might prove useful in further study on X-chromosomal STR polymorphisms. The results of non-labeled quadruplex PCR in groups 1–4 (Table 1) are shown in Fig. S1 in the Electronic Supplementary Material. We determined the genotypes of approximately 100 samples and the sequences of the common alleles for the 16 targeted X-STRs by this method. The repeat structure and our allele designation were further compared with those described in X-STR org (http://www.chrx-str.org) and other reports [19–21] to ascertain whether they matched established allele nomenclature.

Next, the forward primer in each group was labeled with four types of dye: 6-FAM, VIC, NED, and PET for groups 1, 2, 3, and 4, respectively (Table 1). After adjusting the PCR conditions in each group, multiplex PCR of 16 loci was performed. However, 16-locus multiplex PCR produced non-target PCR products which could not be eradicated by adjustment of PCR conditions alone. We had confirmed successful quadruplex PCR in each labeled group (Fig. S1 in the Electronic Supplementary Material), so we conducted octaplex PCR by combining each group with each one of the other three groups. With some combinations, several unexpected products appeared. However, octaplex PCR on the combination of groups 1 and 2 or groups 3 and 4 produced only the expected products, as shown in Fig. 2. Therefore, we typed many samples by these multiplex PCRs. We also typed control reference sample 9947A to compare our results with those of panel cells. Most of the allele types were identical to those described by Szibor et al. [19] (Table 2). However, instead of nine repeats as reported earlier, our sequence data and PCR fragment size for DXS6803 of 9947A showed an extra repeat and another incomplete repeat comprising a 12-repeat TCTA motif and 11.3-repeat motif containing TCA, respectively. Because our results matched the actual repeat size and nomenclature recommended by the ISFG [21], we have shown our results for DXS6803 according to our allele designation in Table 3.

Population studies

We typed 16 X-STR loci (DXS7424, GATA172D05, HPRTB, DXS8377, GATA31Eo8, DXS9895, DXS7423, DXS981, DXS6803, DXS6789, DXS6800, DXS6809, DXS7133, DXS7132, DXS101, and DXS6807) for 339 unrelated male and 173 unrelated female individuals in the Japanese population (Table S1 in the Electronic Supplementary Material). Significant differences were observed in allele frequencies between men and women at DXS6803 and DXS8377 ($P<0.0005$). However, no significant differences were observed at the other loci ($P>0.173$), so the combined allele frequencies for both male and female are shown in Table 3, including those for DXS6803 and DXS8377. Statistical parameters obtained from both men and women are also shown in Table 3. The distribution of allelic frequencies in women was not significantly different from the Hardy–Weinberg Equilibrium ($P>0.017$), except for at DXS6803 and DXS981. Since DXS6803 and DXS981 had alleles with 0.3 and those without 0.3, it is possible that the frequencies and mutation rates in these different types of allele cause disequilibrium at these loci in the Japanese population. Observed heterozygosity in women ranged from 0.933 (DXS6803) to 0.276 (DXS6800). Power of discrimination of the 16 loci ranged from 0.978 (DXS8377) to 0.458 (DXS6800) in females and from 0.897 (DXS8377) to 0.195 (DXS6800) in males. Although the order of the degree of diversity values differed depending on the parameters, many loci, apart from DXS6800 and DXS7133, were fairly informative in the Japanese population. The combined power of discrimination of the 16 loci was 0.999999999999997 in females and 0.9999999992 in males.

Twelve of the 16 loci (DXS7424, GATA172D05, HPRTB, DXS8377, GATA31E08, DXS7423, DXS981, DXS6789, DXS7133, DXS7132, DXS101, and DXS6807) in the present study have been examined in other Japanese populations [7, 22, 23]. The allele frequencies of the present data showed no significant differences to those of these earlier reports ($P>0.015$). Four other loci—DXS9895, DXS6803, DXS6800 and DXS6809—have not

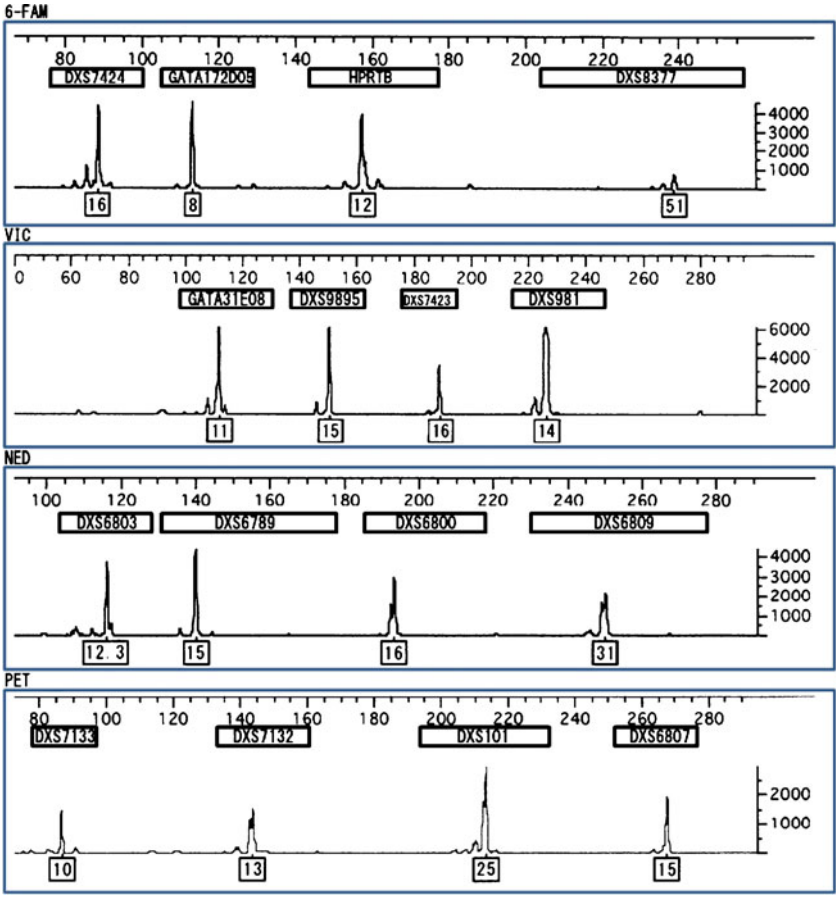

Fig. 2 Electrophoretic profiles obtained by two octaplex PCRs of X-chromosomal STRs. Group 1 (*6-FAM*) and group 2 (*VIC*) were amplified in a single PCR and groups 3 (*NED*) and 4 (*PET*) in other single PCRs

yet been examined in the Japanese population, so we compared the allelic frequencies of these loci with those in the nearest population, the Korean [2, 24–26]. A significant difference was observed only at DXS7132 (P=0.00019).

Linkage equilibrium analysis

The exact test for linkage equilibrium was performed for all pairs of loci in this study. Although some of the loci are closely linked in physical distance, significant deviation was found only between GATA31E08 and DXS101 (P=0.003), DS8377 and DXS6809 (P=0.006), and DS9895 and DXS6807 (P=0.009), which are not physically closely linked with each other. As no real linkage disequilibrium was expected to exist, it is possible that these associations were the result of the sampling effect. A haplotype cluster comprising DXS6801, DXS6809, and DXS6789 has been reported [27]. Forensic tests for DXS6809 and DXS6789 in African-Americans have also suggested they comprise a haplotype rather than independent loci [28]. However, since the P values do not stand after Bonferroni's correction (P< 0.0011), this haplotype cluster has not yet been well established. Nevertheless, linkage disequilibrium between DXS101 and DXS7424 has been described [29]. Although recent studies [28, 30–32] showed no linkage disequilibrium among the loci presented in this study, the high mutation rate of STRs remains to be considered. To allow future comparisons and sample size enlargement, the haplotype frequencies between DXS6803 and DXS6789, DXS7424 and DXS101, and DXS8377 and DXS7423, which are located within 144–794 kbps in physical distance, are shown in Table S2 in the Electronic Supplementary Material.

Development of single multiplex system

In order to effectively obtain more information in a single PCR reaction and apply X-STR polymorphisms to samples with a limited volume in forensic cases, we determined whether the 16-locus multiplex profile could be improved upon. In 16-locus multiplex PCR, extra peaks with lengths of 130, 165, 191, 198, 247, and 277 bps appeared in VIC-labeled PCR, and peaks with lengths of 135 and 165 bps and sometimes 107 bps appeared in NED-labeled PCR (Fig. S2 in the Electronic Supplementary Material). In further experiments, the following products were found when the DXS9895 primer was used: extra peaks with lengths of 130, 165, 191, and 198 bps in VIC-labeled products and 107, 135, and 165 bps in NED-labeled products appeared with multiplex PCR in combination with

Table 2 Allele frequencies in the Japanese population, I

Allele	DXS7424	GATA172D05	HPRTB	DXS8377	GATA31E08	DXS9895	DXS7423	DXS981
6	–	0.085	–	–	–	–	–	–
7	–	0.003	–	–	0.099	–	–	–
8	–	0.112	–	–	0.013	–	–	–
9	–	0.057	–	–	0.190	–	–	–
10	–	0.393	0.001	–	0.279	–	–	–
10.3	–	–	–	–	–	–	–	0.002
11	–	0.280	0.039	–	0.307	–	–	0.005
11.3	–	–	–	–	–	–	–	0.010
12	0.025	0.070	0.292	–	0.100	0.003	–	0.035
12.3	–	–	–	–	–	–	–	0.053
13	0.029	–	0.440	–	0.010	0.250	0.005	0.215
13.3	–	–	–	–	–	–	–	0.093
14	0.166	–	0.148	–	0.001	0.294	0.328	0.301
14.3	–	–	–	–	–	–	–	0.040
15	0.288	–	0.055	–	–	0.239	0.608	0.190
15.3	–	–	–	–	–	–	–	0.026
16	0.445	–	0.025	–	–	0.190	0.060	0.041
16.3	–	–	–	–	–	–	–	0.003
17	0.037	–	–	–	–	0.021	–	0.003
18	0.011	–	–	–	–	0.003	–	–
41	–	–	–	0.001	–	–	–	–
42	–	–	–	0.009	–	–	–	–
43	–	–	–	0.031	–	–	–	–
44	–	–	–	0.043	–	–	–	–
45	–	–	–	0.058	–	–	–	–
46	–	–	–	0.105	–	–	–	–
47	–	–	–	0.135	–	–	–	–
48	–	–	–	0.108	–	–	–	–
49	–	–	–	0.156	–	–	–	–
50	–	–	–	0.103	–	–	–	–
51	–	–	–	0.085	–	–	–	–
52	–	–	–	0.055	–	–	–	–
53	–	–	–	0.043	–	–	–	–
54	–	–	–	0.024	–	–	–	–
55	–	–	–	0.024	–	–	–	–
56	–	–	–	0.010	–	–	–	–
57	–	–	–	0.004	–	–	–	–
58	–	–	–	0.003	–	–	–	–
NA9947A	14, 16	10	15	45, 47	11	13, 17	14, 15	13.3, 14.3
P	0.017	0.561	0.339	0.052	0.735	0.324	0.451	0.003
Hobs	58.4	77.1	72.8	88.0	78.3	75.0	53.3	70.5
PDf	0.856	0.884	0.844	0.978	0.906	0.893	0.652	0.935
PDm	0.685	0.738	0.701	0.897	0.775	0.757	0.541	0.813
PE	0.273	0.547	0.473	0.754	0.568	0.510	0.218	0.433
PIC	0.64	0.70	0.64	0.90	0.73	0.71	0.42	0.79

P values of exact tests for Hardy–Weinbelg equilibrium, *Hobs* observed heterozygosity, *PDf*: power of discrimination in women, *PDm* power of discrimination in men. PE: Power of Exclusion, *PIC* polymorphism information content

Table 3 Allele frequencies in the Japanese population, II

Allele	DXS6803	DXS6789	DXS6800	DXS6809	DXS7133	DXS7132	DXS101	DXS6807
9.0	–	–	–	–	0.700	–	–	–
10.0	0.014	–	–	–	0.259	–	–	–
10.3	0.006	–	–	–	–	–	–	–
11.0	0.165	–	–	–	0.035	0.003	–	0.370
11.3	0.162	–	–	–	–	–	–	–
12.0	0.185	–	–	–	0.006	0.059	–	0.008
12.3	0.358	–	–	–	–	–	–	–
13.0	0.029	0.002	–	–	–	0.162	–	0.032
13.3	0.067	–	–	–	–	–	–	–
14.0	0.003	0.008	–	–	–	0.380	–	0.367
14.3	0.011	–	–	–	–	–	–	–
15.0	–	0.159	–	–	–	0.286	–	0.193
15.3	0.002	–	–	–	–	–	–	–
16.0	–	0.313	0.869	–	–	0.096	–	0.029
17.0	–	0.017	–	–	–	0.013	–	0.002
18.0	–	0.002	–	–	–	–	–	–
19.0	–	0.024	0.086	–	–	–	–	–
20.0	–	0.174	0.002	–	–	–	–	–
21.0	–	0.179	0.006	–	–	–	0.008	–
22.0	–	0.085	0.038	–	–	–	0.041	–
23.0	–	0.036	–	–	–	–	0.131	–
24.0	–	0.003	–	–	–	–	0.305	–
25.0	–	–	–	–	–	–	0.198	–
26.0	–	–	–	0.006	–	–	0.179	–
27.0	–	–	–	–	–	–	0.087	–
28.0	–	–	–	–	–	–	0.033	–
29.0	–	–	–	0.003	–	–	0.014	–
30.0	–	–	–	0.029	–	–	0.003	–
31.0	–	–	–	0.149	–	–	0.002	–
32.0	–	–	–	0.163	–	–	–	–
33.0	–	–	–	0.309	–	–	–	–
34.0	–	–	–	0.205	–	–	–	–
35.0	–	–	–	0.090	–	–	–	–
36.0	–	–	–	0.038	–	–	–	–
37.0	–	–	–	0.006	–	–	–	–
NA9947A	11.3, 12	21, 22	18, 19	31, 34	9, 10	12	24, 26	12, 14
P	0.005	0.221	0.158	0.047	0.298	0.807	0.463	0.096
Hobs	93.3	74.7	27.6	77.5	43.6	71.2	77.6	64.8
PDf	0.873	0.933	0.458	0.928	0.636	0.876	0.931	0.857
PDm	0.747	0.805	0.195	0.804	0.421	0.733	0.816	0.686
PE	0.862	0.504	0.054	0.533	0.138	0.447	0.556	0.352
PIC	0.77	0.78	0.26	0.78	0.39	0.68	0.77	0.64

P values of exact tests for Hardy–Weinbelg equilibrium, *Hobs* observed heterozygosity, *PDf*: power of discrimination in women, *PDm* power of discrimination in men. PE: Power of Exclusion, *PIC* polymorphism information content

DXS9895 and DXS6800, and peaks with lengths of 247 and 277 bps in NED-labeled products appeared in combination with DXS9895 and DXS101 (Fig. S3 in the Electronic Supplementary Material). With in silico PCR (http://www.genome.ucsc.edu), the DXS9895 and DXS6800 primers yielded no products corresponding to other extra peaks. In order to determine the origin of the extra peaks, we tried to sequence 130-, 135-, 165-, 192-,

and 198-bp bands after re-amplification from the gel. The 130-bp product was successfully sequenced as a single sequence and corresponded to a 136-bp region on chromosome 13. The DXS9895 forward primer and 17 nucleotides of the 3′-side of the DXS6800 forward primer completely matched this region. Because both the sequence of the 105-bp region including the 9895-F primer and the sequence of the 63-bp region including the 6800-F primer were almost identical between chromosome X and chromosome 13, it was difficult to construct primers outside of these regions in our multiplex system. Sequencing of other bands with sizes of 135, 165, 192, and 198 bp was unsuccessful due to the mixed profile in the sequence electrophoretograms. Although partially incomplete sequences were obtained in some samples, they corresponded to the common sequence found in various autosomal chromosomes. In the experiments described above, we used the same DXS6800 primer pair as described elsewhere [1]. Under the octaplex PCR conditions described in the Materials and methods section, the above-mentioned 107-bp product in NED was not amplified. However, it was amplified in octaplex PCR under the same PCR conditions 1 year after preparation of the 6800 primer stocks, probably due to degradation of the primer. Therefore, we moved the region amplified by the forward and reverse primers by as many as 16 bps in the 3′-end direction (Table 1). As a result, the DXS6800 forward primer did not match the counterpart of a 110-bp sequence on chromosome 9, which resulted in loss of the 107-bp band found in the allelic region of DXS6800. The forward and reverse primers of DXS9895 and DXS6800 have many counterparts on autosomal chromosomes. There is the possibility that these primers will produce unwanted sequences in PCR if the annealing/extension temperature is low. After several trials to improve the electrophoretograms, we concluded that it was very difficult to change the primers of DXS9895 and DXS6800 appropriately under the multiplex PCR conditions described in this paper. Finally, we applied multiplex PCR of the present 15 loci, exclusive of the DXS9895 locus, in a single reaction (Fig. 3). This multiplex system produced none of the extra peaks described above and worked well in typing of X-STRs from degraded DNAs extracted from old skeletal remains in our routine forensic cases. It amplified the largest number of loci among the X-STR multiplex systems used and

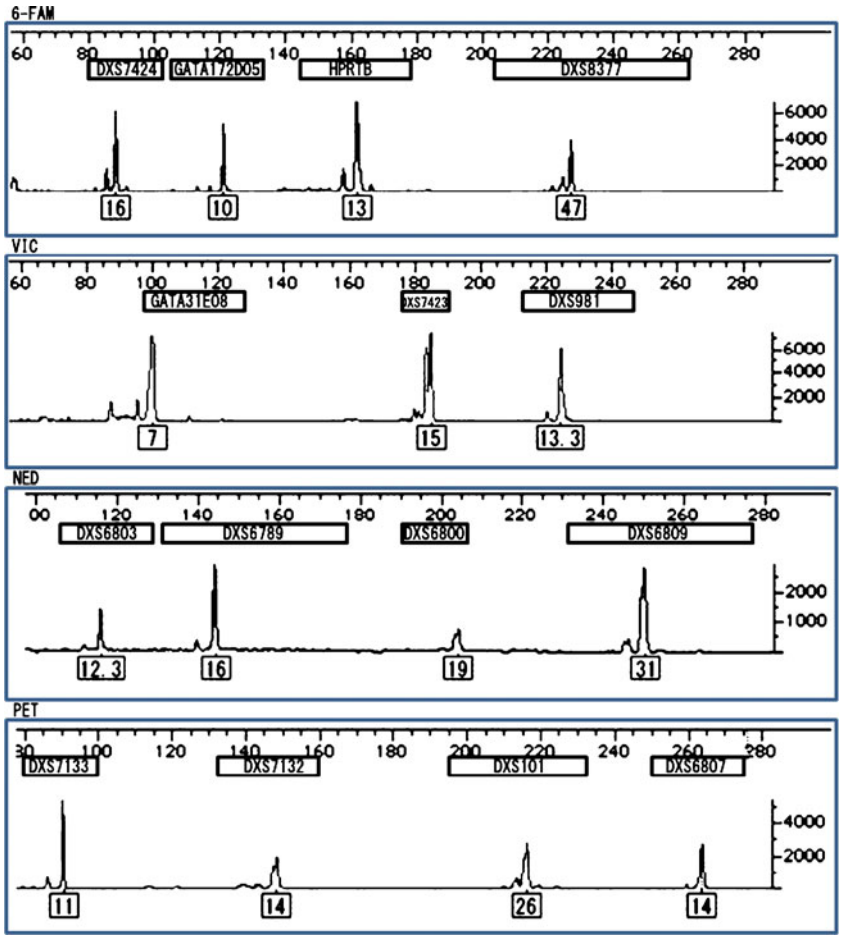

Fig. 3 Electrophoretic profiles obtained by 15-locus multiplex PCR. No extra peaks interfered with typing of 15 loci, and reliable typing was possible

afforded a power of discrimination in the order of 0.99999999999997 in females and 0.999999997 in males in the Japanese population, although this was one digital lower that that obtained with 16-locus multiplex data. It is especially useful to be able to inspect a large number of loci over the entire X chromosome in a single PCR reaction when we need to determine whether a part of the X chromosome has been inherited in a complex case.

In conclusion, we developed two kinds of octaplex PCR system for X-STRs and analyzed a large number of individuals in the Japanese population. We then further developed a 15-locus multiplex system excluding the DXS9895 locus. The flanking regions of the DXS9895 STR locus have many counterparts on various chromosomes and produce extra products in combination with DXS6800 or DXS101. The results of this study indicate that care must be taken in constructing X-STR multiplex systems including the DXS9895 locus.

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