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SHORT COMMUNICATION

Thirteen X-chromosomal short tandem repeat loci multiplex data from Taiwanese

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Abstract Study results of variations in the X chromosome are useful tools in researching the genetic diversity of human populations and individual identification. We developed a 13 X chromosomal short tandem repeat (STR) multiplex system (DXS6807, DXS8378, DSX9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7424, DXS101, GATA172D05, HPRTB, DXS8377, DXS7423) amplified in one single polymerase chain reaction. DNA samples of 113 male and 108 female Taiwanese Han subjects were successfully analyzed using this 13 X-STR multiplex system. The distributions of allele frequencies were examined for independence. DXS8377, DXS101, DXS6789, and DXS6809 were found to be the most polymorphic markers in this study. High values of discrimination power and mean exclusion chance without significant evidence of association between these markers were obtained. In conclusion, this 13 X chromosomal STR multiplex system offers considerable forensic efficiency and may be useful in forensic identification casework.

Keywords Forensic identification · Multiplex PCR · Short tandem repeat · X chromosome

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Introduction

The reference sequences for the X chromosome were completed in 2005 [1] and provide common reference data for investigations of X chromosome genetic disorders and anthropological studies. The X chromosome is present in the population at three-quarters of the level of any autosomal chromosome and lacks recombination with the Y chromosome. Therefore, a reduced X chromosome variation is expected. The variation of the X chromosome has been observed to be about 60% of the autosomal level [2]. Human variation in the X chromosome is an invaluable tool for researching the genetic diversity of human populations and individual identification. In spite of their relatively lower degree of variability in comparison to autosomal chromosomes, X chromosome short tandem repeats (STRs) may complement the analysis of Y and autosomal STR data. In cases of female offspring with an unavailable alleged father, the X chromosomes of close paternal relatives have to be analyzed.

The X chromosome has a sex-based mode of inheritance. Because the X chromosome recombines only in females, there is a smaller effective population size, a stronger genetic drift, and higher linkage disequilibrium. These factors facilitate the X chromosome in being a source of information in human evolution and migration studies [1]. However, a limited number of X chromosome STR polymorphisms have been reported [3–12]. Gomes et al. have

described an X-STR decaplex system [11], and based on the results of their report, we developed a 13 X-STR multiplex system that could be amplified in one single polymerase chain reaction (PCR).

This study presents information of this partially newly established 13 X-chromosomal fluorescent STR loci multiplex system and its application to the analysis of a Taiwanese Han population. The haplotypes and distribution of allele frequencies of these 13 X-STR markers are demonstrated. We also assess the association between these X-STR markers and evaluate their patterns of polymorphisms for possible forensic identification purposes (Fig. 1).

Materials and methods

Sample sources and DNA extraction

This retrospective study was approved by the Institution Review Board. A total of 221 DNA samples from 113 male and 108 female apparently healthy unrelated Taiwanese Han subjects were analyzed. The blood samples and buccal swab samples were obtained from volunteer donors, with informed consent, between 1993 and 2007. Standard methods of phenol-chloroform/isoamyl alcohol extraction and the QIAamp blood kit (Qiagen, Hilden, Germany) were used for DNA extraction from peripheral whole blood samples, and the Blood & Tissue Genomic DNA extraction Miniprep system (Viogene, Taipei, Taiwan) was used for DNA extraction from buccal cells. The amount of extracted DNA was quantitated with a UV spectrophotometer (Biowave S2100, Life Science, Cambridge, UK).

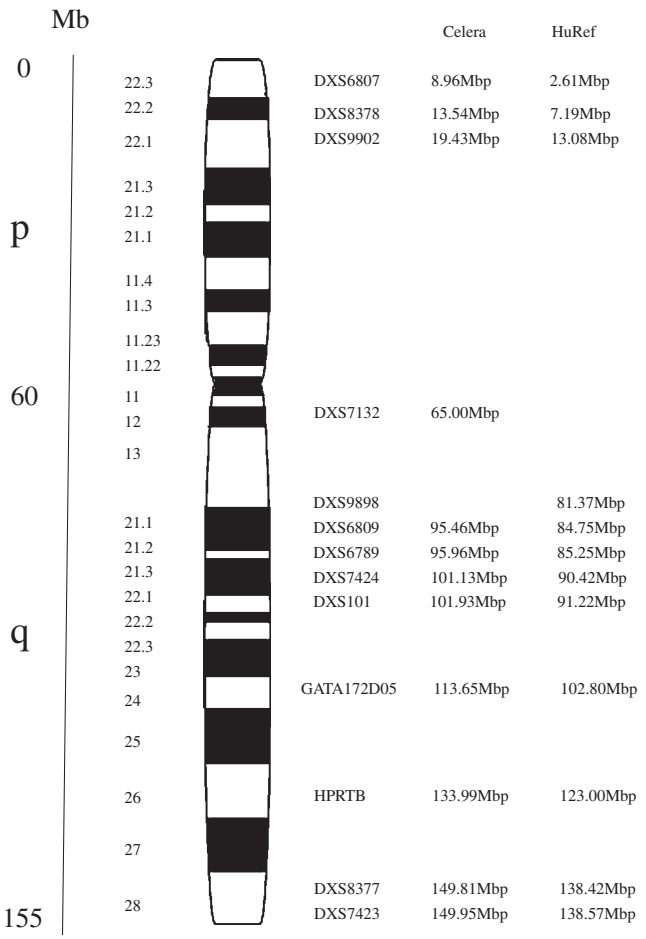

Fig. 1 Location of the 13 short tandem repeat loci studied on the X chromosome

X chromosome marker typing

One multiplex PCR was performed with the partially newly designed primer sets. Nine pairs of primer sequences were identical to the primers described in previous papers [3, 5, 11, 13]. For DXS6807, DXS9902, DXS7132, and DXS7424, new primers were designed using PRIMER3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The 13 X-STRs (DXS6807, DXS8378, DSX9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7424, DXS101, GATA172D05, HPRTB, DXS8377, DXS7423) and amelogenin were typed following the methodology of Gomes et al. with minimal modifications [11]. Table 1 lists the primer sequences and dye labels used. Briefly, PCR reactions were performed in a total volume of 10 μL containing 1 ng of genomic DNA, 1× Super-Therm PCR buffer (JMR Holdings, Sevenoaks, UK), 1.5 mM $MgCl_2$, 250 μM of each deoxyribonucleotide triphosphate, primer sets, and 0.5 U of Super-Therm Gold DNA polymerase (JMR Holdings). The amount of each primer set in multiplex PCR mixture was listed in Table 1. PCR was performed using a GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) in 9600 mode. The cycling programs consisted of predenaturation at 95°C for 11 min, followed by 30 cycles of denaturing at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and a final extension at 72°C for 60 min.

Electrophoresis was performed using an ABI 3100 Genetic Analyzer (Applied Biosystems) in which 1 μl of multiplex PCR product was mixed with 10 μl Hi-Di formamide and 0.2 μl of the GeneScan-500LIZ internal size standard. Fragment sizes were automatically determined using GeneScan Analysis software (Applied Biosystems). Genotyping was analyzed using either Genotyper or GeneMapper ID software (Applied Biosystems) by comparison with reference DNA control samples 9947A (female) (Applied Biosystems) and K562 (male) (Promega, Madison, WI, USA).

The DNA samples with new alleles detected were sequenced using the ABI Big Dye Terminator Cycle Sequencing Ready Kit (Applied Biosystems). The product

Table 1 Primer sequences, labels, references and amount in PCR reaction used in this study

X-STR loci	Dye label	Primer sequences Forward primer (5'-3') Reverse primer (5'-3')	Amount μM	Reference
DXS6807F	PET	GGAGAGCAATGATCTCATTTGCA	1.5	This study
DXS6807R		TAAGTAAACATGTATAGGAAAAAGCTAC		
DXS8378F	6-FAM	TTAGGCAACCCGGTGGTCC	0.38	[3]
DXS8378R		GACAAGAACGAAACTCCAACTC		
DXS9902F	PET	AATGGAGTCTCTGGGTGAAGAG	0.96	This study
DXS9902R		CATATCAGGAGTATGGGATCACC		
DXS7132F		AATCCCCTCTCATCTATCTGAC	0.71	This study
DXS7132R	NED	CACTCCTGGTGCCAAACTCT		
DXS9898F	6-FAM	CGAGCACACCTACAAAAGCTG	0.27	[11]
DXS9898R		GCTTCTTAGGCTCACCTCACTGAGCA		
DXS6809F	VIC	TCCATCTTTCTCTGAACCTTCC	0.53	[11]
DXS6809R		GCTTCTTGCTTTAGGCTGATGTGAGG		
DXS6789F	NED	CTTCATTATGTGCTGGGGTAAA	0.71	[11]
DXS6789R		ACCTCGTGATCATGTAAGTTGG		
DXS7424F	PET	GGTTCATAGTGTCAGATAACTGAG	1.44	This study
DXS7424R		TATGCCAGCCACTGGACCTG		
DXS101F	NED	GTTTTATCCCCGCTACAGGA	0.71	[11]
DXS101R		CTGCATATTCTGCGCATGT		
GATA172D05F	VIC	TAGTGGTGATGGTTGCACAG	0.53	[3]
GATA172D05R		ATAATTGAAAGCCCGGATTC		
HPRTB-F		CTCTCCAGAATAGTTAGATGTAGG	0.7	[5]
HPRTB-R	6-FAM	ATGCCACAGATAATACACATCCCC		
DXS8377F	6-FAM	ACCACTTCATGGCTTACCACAG	0.47	[5]
DXS8377R		GTATGGACCTTTGGAAAGCTAG		
DXS7423F	VIC	GTCTTCCTGTCATCTCCCAAC	0.45	[3]
DXS7423R		GCTTCTTAGCTTAGCGCCTGGCACATA		
AMEL-F	PET	CCCTGGGCTCTGTAAAGAATAGTG	0.65	[13]
AMEL-R		ATCAGAGCTTAAACTGGGAAGCTG		

was detected in the ABI PRISM 3100 Genetic Analyzer electrophoresis system and analyzed with sequencing analysis 3.7 software (Applied Biosystems).

Statistical analysis

Linkage disequilibrium and Hardy–Weinberg equilibrium analysis by the exact test were performed using the GENEPOP (version 3.4) software package [14].

Results and discussion

The 13 X-linked STR markers were successfully amplified in one single PCR multiplex reaction. Allelic nomenclature used for genotyping for most loci followed the description by Szibor et al. [15]. No identical haplotype-like allelic combinations of 13 X-STR markers were found in the 113 unrelated males in this study.

X-STR alleles

Table 2 shows the allele frequencies and discrimination powers obtained for the 13 X-STR loci (DXS6807, DXS8378, DSX9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7424, DXS101, GATA172D05, HPRTB, DXS8377, DXS7423) studied in both male and female Taiwanese Han subjects. The distributions of allele frequencies in the male and female subjects were examined using the χ^2 test for independence. The p values of the 13 loci showed no significant differences ($p>0.05$). Among these data, two alleles of DXS8377 have not been reported previously [3, 11, 16–18]. These two new alleles were sequenced for confirmation and the results are presented in Table 3.

Forensic efficiency

Table 2 presents the forensic statistical evaluation parameters calculated for the Taiwanese. Among these 13 loci,

Table 2 Allele frequencies and discrimination power for the 13 X chromosomal short tandem repeat markers used in this study

Allele	DXS6807	DXS8378	DXS9902	DXS7132	DXS9898	DXS6789	DXS7424	DXS101	GATA 172D05	HPRTB	DXS7423	Allele	DXS6809	DXS8377
6									0.0486			28	0.0030	
7			0.0030						0.0030			29	0.0091	0.0030
8									0.1915			30	0.0122	
8.3					0.0182							31	0.1459	
9		0.0213	0.0091						0.1185			32	0.1611	
10		0.5106	0.4590		0.0030		0.0091		0.3891			33	0.2523	
11	0.4438	0.3161	0.2796	0.0030	0.0669				0.2006	0.0699		34	0.1854	0.0030
12	0.0122	0.1185	0.2310	0.0942	0.5623				0.0486	0.2918		35	0.1429	
13	0.0061	0.0213	0.0182	0.1976	0.2644		0.0426			0.3982	0.0030	36	0.0608	
14	0.3313	0.0122		0.3587	0.0790	0.0061	0.1185			0.1824	0.3495	37	0.0243	
15	0.1611			0.2827	0.0061	0.1793	0.3678			0.0456	0.5775	38	0.0030	
16	0.0426			0.0486		0.2796	0.3891			0.0122	0.0699	42		0.0030
17	0.0030			0.0152		0.0578	0.0669					43		0.0274
18						0.0061	0.0061					44		0.0456
19						0.0152		0.0030				45		0.0638
20						0.1854		0.0030				46		0.0942
21						0.1581		0.0091				47		0.1398
22						0.0760		0.0486				48		0.1216
23						0.0274		0.1368				49		0.1368
24						0.0061		0.3191				50		0.0942
25						0.0030		0.1550				51		0.0821
26								0.1611				52		0.0547
27								0.0699				53		0.0669
28								0.0608				54		0.0334
29								0.0213				55		0.0122
30								0.0091				56		0.0061
31								0.0030				57		0.0030
												58		0.0091
PD_F	0.8296	0.7802	0.8020	0.8954	0.7968	0.9408	0.8486	0.9408	0.8954	0.8596	0.6977		0.9408	0.9755
PD_M	0.6405	0.6433	0.6395	0.7365	0.5944	0.8272	0.6806	0.8145	0.7767	0.7354	0.5358		0.8214	0.9019
PE	0.4200	0.3400	0.4790	0.4490	0.2710	0.6620	0.4940	0.5100	0.5100	0.4340	0.2310		0.8110	0.8110
MEC	0.4023	0.3627	0.3838	0.5101	0.3593	0.6462	0.4457	0.6503	0.5395	0.4755	0.2598		0.6605	0.8110
PIC	0.6595	0.5690	0.6160	0.7093	0.5549	0.8125	0.6468	0.8075	0.7353	0.6774	0.4546		0.8194	0.9046
Het_{Obs}	0.6944	0.6389	0.7315	0.7130	0.5833	0.8333	0.7407	0.7500	0.7500	0.7037	0.5463		0.9074	0.9074
P_{HW}	0.7717	0.7102	0.2200	0.7399	0.8104	0.9891	0.9094	0.2184	0.8354	0.7124	0.8216		0.7348	0.5857

PD_F power of discrimination in females, *PD_M* power of discrimination in males, *PE* power of exclusion, *MEC* mean exclusion chance, *PIC* polymorphism information content, *Het_{Obs}* observed heterozygosity, *P_{HW}* probability values of exact tests for Hardy-Weinberg equilibrium

Table 3 Sequencing results of new alleles for DXS8377

Sample	Allele	Sequence structure
110091	29	$(AGA)_{29}$
120056	34	$(AGA)_{15}(GGA\text{-}AGA)_5(AGA)_2GGA(AGA)_6$

DXS8377 was found to be the most polymorphic, followed by DXS101, DXS6789, and DXS6809. The results of the two most polymorphic markers, DXS8377 and DXS101, were similar to previous reports from African-Americans and Caucasians [11–12, 16]. However, the results were different from a report with the second most polymorphic marker, DXS6809, for Asians [11], and there may be discrepancies among different Asian populations. The least discriminating locus was DXS7423, with only four alleles found in this study, which was consistent with previous reports [11, 12]. The combined power of exclusion and combined mean exclusion chance were 0.999949 and 0.999942, respectively. The combined power of discrimination in females and males was $1–5.38\times10^{-13}$ and $1–2.43\times10^{-8}$, respectively. The mean exclusion chance resulted in a high value in this population (Table 2), which supports the potential of this 13 STR multiplex system to serve in a specific kinship analysis context when father–daughter relationships are investigated. Since high values for discrimination power were obtained from this X chromosome STR multiplex, this system can be used in forensic identity testing.

For all pairs of the loci, the exact test for linkage disequilibrium was performed. The p values ranged from 0.9886±0.0040 to 0.0389±0.0136 (between DXS6807 and DXS9902), which was consistent with previous reports of no significant linkage disequilibrium [11, 12].

A haplotype cluster group with DXS6801, DXS6809, and DXS6789 has been reported [19]. Since the p values do not stand Bonferroni's correction ($p<0.0011$), this haplotype cluster group has not yet been well established. Forensic tests for DXS6809 and DXS6789 in African-Americans have been suggested to be considered as haplotypes instead of independent loci [11]. An association between DXS9898 and DXS6789 has also been reported with a p value of 0.0005 after Bonferroni correction [12]. Nevertheless, linkage disequilibrium between DXS101 and DXS7424 has been described [20]. The haplotype frequencies of the possibly closely linked markers DXS9898–DXS6789, DXS6809–DXS6789, and DXS7424–DXS101 are shown in Table 4. The association between DXS9898 and DXS6789, between DXS6809 and DXS6789, or between DXS7424 and DXS101 was not observed in this dataset. The lack of association between these X-STR loci in this study increases the power of discrimination of this multiplex system. The result of linkage disequilibrium

Table 4 Haplotype frequencies of DXS9898–DXS6789, DXS6809–DXS6789, and DXS7424–DXS101 of 113 males

		Frequency	SE
DXS9898–DXS6789			
8.3	16	0.0088	0.0088
8.3	21	0.0088	0.0088
11	16	0.0177	0.0124
11	17	0.0177	0.0124
11	20	0.0265	0.0151
12	14	0.0088	0.0088
12	15	0.0973	0.0279
12	16	0.1593	0.0344
12	17	0.0531	0.0211
12	20	0.0708	0.0241
12	21	0.1062	0.0290
12	22	0.0354	0.0174
12	23	0.0088	0.0088
13	15	0.0531	0.0211
13	16	0.0796	0.0255
13	17	0.0088	0.0088
13	19	0.0265	0.0151
13	20	0.0619	0.0227
13	21	0.0531	0.0211
13	22	0.0354	0.0174
13	23	0.0088	0.0088
14	15	0.0354	0.0174
14	22	0.0088	0.0088
14	23	0.0088	0.0088
DXS6809–DXS6789			
29	14	0.0088	0.0088
30	17	0.0088	0.0088
31	15	0.0265	0.0151
31	16	0.0177	0.0124
31	17	0.0088	0.0088
31	20	0.0265	0.0151
31	21	0.0354	0.0174
31	22	0.0265	0.0151
32	16	0.0619	0.0227
32	17	0.0265	0.0151
32	19	0.0088	0.0088
32	20	0.0442	0.0193
32	21	0.0531	0.0211
33	15	0.0708	0.0241
33	16	0.0619	0.0227
33	17	0.0265	0.0151
33	19	0.0088	0.0088
33	20	0.0177	0.0124
33	21	0.0265	0.0151
33	22	0.0177	0.0124
33	23	0.0088	0.0088
34	15	0.0177	0.0124
34	16	0.0531	0.0211
34	17	0.0088	0.0088
34	19	0.0088	0.0088
34	20	0.0442	0.0193
34	21	0.0354	0.0174
34	22	0.0088	0.0088
34	23	0.0177	0.0124

Table 4 (continued)

		Frequency	SE
35	15	0.0442	0.0193
35	16	0.0531	0.0211
35	20	0.0177	0.0124
35	21	0.0088	0.0088
35	22	0.0265	0.0151
36	15	0.0177	0.0124
36	16	0.0177	0.0124
36	20	0.0088	0.0088
36	21	0.0088	0.0088
38	15	0.0088	0.0088
DXS7424–DXS101			
13	22	0.0177	0.0124
13	24	0.0265	0.0151
13	25	0.0088	0.0088
14	22	0.0088	0.0088
14	24	0.0442	0.0193
14	25	0.0265	0.0151
14	26	0.0088	0.0088
14	28	0.0088	0.0088
15	23	0.0708	0.0241
15	24	0.1593	0.0344
15	25	0.0442	0.0193
15	26	0.0619	0.0227
15	27	0.0177	0.0124
15	28	0.0265	0.0151
15	29	0.0088	0.0088
16	22	0.0265	0.0151
16	23	0.0619	0.0227
16	24	0.0796	0.0255
16	25	0.0708	0.0241
16	26	0.0619	0.0227
16	27	0.0708	0.0241
16	28	0.0088	0.0088
16	29	0.0088	0.0088
17	22	0.0088	0.0088
17	25	0.0354	0.0174
17	27	0.0177	0.0124
18	23	0.0088	0.0088

SE standard errors

evaluation depends on sample size. Further study with enlargement of sample size is necessary for true linkage disequilibrium information.

In conclusion, this 13 X chromosomal STR multiplex system offers considerable polymorphic patterns in one single reaction. It is useful in forensic identification analysis.

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