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SHORT COMMUNICATION

Development and population study of the 12 X-STR loci multiplexes PCR systems

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Abstract To develop a multiplex polymerase chain reaction (PCR) system with 12 X-chromosomal short-tandem repeat (X-STR) loci and to investigate their polymorphism and linkage and/or independence, the 12 loci (DXS6807, DXS8378, DXS9902, DXS6800, DXS6803, DXS6799, DXS6804, GATA172D05, DXS6854, HPRTB, DXS8377, and DXS7423) were simultaneously analyzed in 1,005 unrelated individuals (574 males and 431 females) from Guangdong Han individuals and Kazakh populations living in China. The allele frequencies and mutation rates were investigated. Allele frequency distribution among different populations was compared. Haplotypes of linkage disequilibrium markers (DXS6807–DXS8378–DXS9902) and linked markers (DXS6804–GATA172D05 and DXS8377–DXS7423) were also reported. A total of 117 alleles, ranging from five to 20 for each locus, were observed in our selected populations. Eight cases with mutation of the selected loci were detected in 9,480 meioses. Pairwise comparisons of allele frequencies distribution showed statistically significant differences at most loci among different populations. Haplotype diversity of linked markers was 0.9404–0.9694. The results indicated that this multiplex system is very useful for forensic analysis and may be complementarities for X-12 kits or X-8 kits in forensic case.

Keywords X-STR · Multiplex PCR · Guangdong Han · Kazakh

Introduction

The multiplex polymerase chain reaction (PCR) system of short-tandem repeat (STR) markers is a common tool used for genetic identity testing in the forensic setting. Many multiplex PCR systems of autosomal STR (AS-STR) and Y chromosomal STR (Y-STR) have been reported, and many commercial kits of the AS-STR and the Y-STR were available. In recent years, there are considerable X-chromosomal STR (X-STR) systems researched in the field of population genetics and forensics [1–6]. Moreover, two kits was available including Mentype® Argus X-8 Kit and Investigator Argus X-12 Kit (Biotype AG, Dresden, German), and there was several reports about Argus X-12 or X-8 Kit haplotype in different populations [7–9]. With the complication of forensic cases, AS-STR and the Y-STR marks as well as these two X-STR Kits were not enough in forensic application. Thus, we developed a multiplex PCR system with 12 X-STR loci including DXS6807(Xp22), DXS8378(Xp22), DXS9902(Xp22), DXS6800(Xq13), DXS6803(Xq21), DXS6799(Xq21), DXS6854(Xq25), HPRTB(Xq26) and two clusters of closely linked markers (each spanning <3 cM): DXS6804–GATA172D05(Xq23) and DXS8377–DXS7423(Xq28) (Table S1 shows the physical localisation of these markers). Allele frequency distribution for most X-STR loci varies with different populations [10, 11]. On the other hand, the use of X-STRs requires a precise knowledge not only of allele and haplotype frequencies, but also of the genetic linkage and linkage disequilibrium (LDE) status among markers [7]. This study described the development and characterization of the 12 X-STR loci multiplex PCR systems and investigated polymorphism and linkage and/or independence of the selected markers in Guangdong Han and Kazakh populations from China.

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Materials and methods

Sample preparation and DNA extraction

Blood samples come from 1,005 unrelated individuals in Guangdong Han and Xinjang Kazakh populations from China. In detail, there were 619 Guangdong Han individuals (398 males and 221 females), and 386 Xinjang Kazakh (176 males and 210 females). There were 310 family trios (father–mother–daughter), 170 family duos (mother–son) and 40 three-generation families (grandmother–father–granddaughter). Parents of the trios and mothers of the duos were included in the unrelated individuals. Informed consent was obtained from all the subjects, and the samples were anonymous before the STR typing was started. Genomic DNA was extracted using Chelex-100 methods [12].

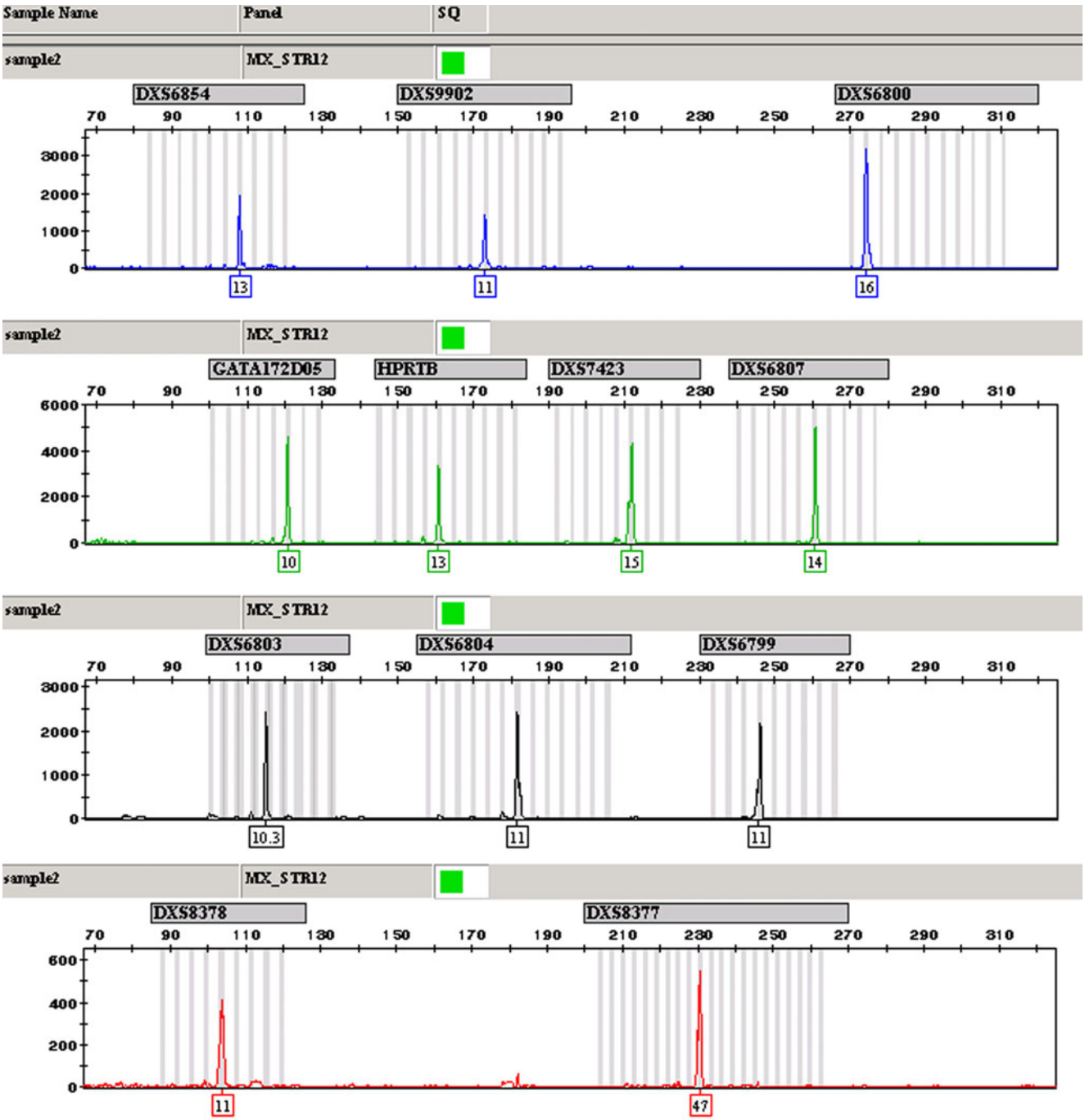

Fig. 1 The electrophoretogram of the twelve X-STR loci typing system

Table 1 Results of pairwise linkage disequilibrium test (significance level=0.05)

Pairs of loci	Exact *P* value	
	Han	Kazakh
DXS6807–DXS8378	0.0237	0.7077
DXS6807–DXS9902	0.0185	0.4133
DXS8378–DXS9902	0.0196	0.9381
DXS6804–GATA172D05	0.0116	0.2969
DXS8377–DXS7423	0.0131	0.5759

No evidence of linkage disequilibrium was detected in Kazakh population

PCR amplification

Amplification was carried out in a 10-μl PCR reaction volume containing 0.5–2 ng DNA, 200 μM for each dNTP, with 1.5 mM $MgCl_2$, 1× buffer, 1.0 U AmpliTaq Gold DNA polymerase (ABI; Foster City, CA, USA). The primer concentration and detail information is presented in Table S1. Samples were amplified in GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 94°C for 11 min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and additional 45 min at 60°C.

Table 2 Haplotype of DXS6807/DXS8378/DXS9902 (N_{Han}=398, N_{Kazakh}=176)

No.	Haplotype	Han		Kazakh		No.	Haplotype	Han		Kazakh	
		Number	Freq.	Number	Freq.			Number	Freq.	Number	Freq.
1	9/10/11	1	0.0025	0		38	14/10/12	12	0.0302	4	0.0227
2	11/9/10	3	0.0075	2	0.0114	39	14/11/7	0		1	0.0057
3	11/9/11	2	0.0050	2	0.0114	40	14/11/9	1	0.0025	1	0.0057
4	11/10/9	1	0.0025	2	0.0114	41	14/11/10	15	0.0377	4	0.0227
5	11/10/10	40	0.1005	15	0.0852	42	14/11/11	23	0.0578	2	0.0114
6	11/10/11	32	0.0804	12	0.0682	43	14/11/12	9	0.0226	3	0.0170
7	11/10/12	13	0.0327	10	0.0568	44	14/12/10	8	0.0201	7	0.0398
8	11/10/13	2	0.0050	0		45	14/12/11	3	0.0075	2	0.0114
9	11/11/7	0		1	0.0057	46	14/13/10	1	0.0025	1	0.0057
10	11/11/10	32	0.0804	9	0.0511	47	14/13/11	0		1	0.0057
11	11/11/11	18	0.0452	11	0.0625	48	14/14/10	1	0.0025	0	
12	11/11/12	8	0.0201	6	0.0341	49	15/9/10	2	0.0050	1	0.0057
13	11/12/9	2	0.0050	1	0.0057	50	15/9/12	2	0.0050	0	
14	11/12/10	19	0.0477	6	0.0341	51	15/10/9	1	0.0025	0	
15	11/12/11	3	0.0075	1	0.0057	52	15/10/10	10	0.0251	0	
16	11/12/12	7	0.0176	5	0.0284	53	15/10/11	8	0.0201	5	0.0284
17	11/13/10	1	0.0025	1	0.0057	54	15/10/12	11	0.0276	3	0.0170
18	11/13/11	1	0.0025	0		55	15/11/10	6	0.0151	2	0.0114
19	11/14/10	1	0.0025	0		56	15/11/11	2	0.0050	3	0.0170
20	12/10/11	3	0.0075	1	0.0057	57	15/11/12	2	0.0050	1	0.0057
21	12/11/10	1	0.0025	1	0.0057	58	15/12/9	2	0.0050	0	
22	12/11/11	1	0.0025	0		59	15/12/10	4	0.0101	2	0.0114
23	12/12/10	1	0.0025	0		60	15/12/11	0		5	0.0284
24	12/12/11	1	0.0025	0		61	15/12/12	1	0.0025	1	0.0057
25	13/10/10	1	0.0025	1	0.0057	62	15/13/12	0		1	0.0057
26	13/10/11	2	0.0050	2	0.0114	63	16/10/10	1	0.0025	3	0.0170
27	13/10/12	1	0.0025	0		64	16/10/11	1	0.0025	2	0.0114
28	13/11/10	1	0.0025	1	0.0057	65	16/10/12	1	0.0025	1	0.0057
29	13/11/11	1	0.0025	1	0.0057	66	16/11/10	0		2	0.0114
30	13/11/12	0		1	0.0057	67	16/11/11	2	0.0050	1	0.0057
31	13/12/10	2	0.0050	1	0.0057	68	16/11/12	1	0.0025	0	
32	13/12/11	0		4	0.0227	69	16/12/10	1	0.0025	1	0.0057
33	13/13/11	0		1	0.0057	70	16/12/11	1	0.0025	0	
34	14/9/10	1	0.0025	0		71	17/10/11	0		1	0.0057
35	14/10/9	0		1	0.0057	72	17/11/10	0		1	0.0057
36	14/10/10	39	0.0980	9	0.0511	73	17/12/10	1	0.0025	1	0.0057
37	14/10/11	24	0.0603	5	0.0284						
Haplotype diversity									0.9508		0.9694

Sample electrophoresis and data analysis

PCR products were separated by capillary electrophoresis using ABI PRISM 3100 Genetic Analyzer with denaturing polymer 3100 POP-4™ (Applied Biosystems) with GeneMapper ID 3.1 Analysis Software. Fragment sizing was supported using the Genescan™-500 LIZ™ size standards. Allele typing was based on home-made allelic ladder, and the K562, and 9947A (Promega Corporation, Madison, WI, USA) cell lines DNA were typed for calibrating allelic ladder.

Sensitivity testing

K562 cell DNA was diluted with quantities of 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.06 ng to perform sensitivity experiment, and each level of DNA was amplified with the multiplex system, respectively.

Sequence analysis

PCR product was purified or cloned by TOP10F Cloning Kit following the manufacturer's instructions. Then, purified PCR product or the chosen clones was sequenced on ABI 3100 Genetic Analyzer using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions.

Statistical analysis

The software ARLEQUIN 3.5 [13] was used to perform the following statistical analysis, including allelic frequencies and haplotype frequencies, the exact test for HWE for female data, LDE test between all pairs of markers. The exact test differentiation of allele frequency distribution among different populations was performed with SPSS v16.0. Polymorphism information content (PIC) was estimated according to Botstein et al [14]. The power of discrimination in females (PD_F) and males (PD_M), mean exclusion chance (MEC) were calculated according to Desmarais et al. [15].

Results and discussion

A new multiplex system with the 12 markers was successfully developed in this study (Fig. 1). DNA of 8, 4, 2, 1, 0.5, 0.25 and 0.125 ng was successful analyzed using this multiplex system. In forensic practice, we routinely used about 0.5–2 ng DNA, although 0.1 ng DNA is enough for typing. Repeated analysis of random DNA samples had consistent results. The results of K562, and 9947A control DNA calibrating allelic ladder were in agreement with those reported by Szibor et al. [16]. Genotypes of (14) and (13, 15) were observed for K562 and 9947A DNA at DXS6804, and genotypes of (11) and (11, 12) were observed for K562

Table 3 Haplotype of DXS6804 and GATA172D05 (N_{Han}=398, N_{Kazakh}=176)

No.	Haplotype	Han		Kazakh		No.	Haplotype	Han		Kazakh	
		Number	Freq.	Number	Freq.			Number	Freq.	Number	Freq.
1	8/8	0		1	0.0057	19	13/8	26	0.0653	8	0.0455
2	8/9	2	0.0050	0		20	13/9	18	0.0452	1	0.0057
3	10/10	0		1	0.0057	21	13/10	55	0.1382	23	0.1307
4	11/6	3	0.0075	2	0.0114	22	13/11	34	0.0854	13	0.0739
5	11/7	5	0.0126	0		23	13/12	6	0.0151	5	0.0284
6	11/8	28	0.0704	3	0.0170	24	14/6	4	0.0101	3	0.0170
7	11/9	5	0.0126	3	0.0170	25	14/8	16	0.0402	4	0.0227
8	11/10	22	0.0553	22	0.1250	26	14/9	13	0.0327	3	0.0170
9	11/11	17	0.0427	9	0.0511	27	14/10	29	0.0729	11	0.0625
10	11/12	6	0.0151	4	0.0227	28	14/11	18	0.0452	3	0.0170
11	12/6	5	0.0126	5	0.0284	29	14/12	1	0.0025	2	0.0114
12	12/8	11	0.0276	9	0.0511	30	15/8	9	0.0226	0	
13	12/9	9	0.0226	1	0.0057	31	15/9	2	0.0050	2	0.0114
14	12/10	24	0.0603	12	0.0682	32	15/10	3	0.0075	4	0.0227
15	12/11	11	0.0276	4	0.0227	33	15/11	6	0.0151	1	0.0057
16	12/12	1	0.0025	2	0.0114	34	15/12	1	0.0025	1	0.0057
17	13/6	6	0.0151	13	0.0739	35	16/10	0		1	0.0057
18	13/7	1	0.0025	0		36	16/11	1	0.0025	0	
Haplotype diversity									0.9421		0.9404

and 9947A DNA at DXS6799, which have not been reported in earlier studies.

Hardy–Weinberg equilibrium (HWE) was performed on female samples, and the genotype distributions did not deviate from HWE at the 12 loci. Allele frequencies between female and male samples were not significantly different in all the examined loci. The allele frequencies and further statistical information of the 12 loci in the two population groups are listed in Table S2. PIC of all of selected loci reached above 0.61 with exception of DXS6800 and DXS7423, as well as DXS6799 in Han population. In particular, the PIC of DXS8377 loci went beyond 0.90. PD_F of the loci exceeded 0.98. DXS8377 loci were highly polymorphic, with the very high power of discrimination and probability of paternity exclusion among the loci studied. Eight cases of mutation were detected from the 12 loci in 9,480 meioses. Mutation information is listed in Table S3. All the mutations were the shift of one repeat unit. The average mutation rate for the 12 loci was estimated to be 0.996×10^{-3} per meiosis. These results suggest that the 12 X-STR loci system has sufficient forensic efficiency.

DXS6804–GATA172D05 and DXS8377–DXS7423 can be regarded as linkage groups for their genetic distance spanned <3 cM. Furthermore, the exact test for LDE was performed for these pairs of loci two populations. *P* values of the exact test for LDE are listed in Table 1. When LDE

Table 4 Haplotype of DXS8377 and DXS7423 (N_{Han}=398, N_{Kazakh}=176)

No.	Haplotype	Han		Kazakh		No.	Haplotype	Han		Kazakh	
		Number	Freq.	Number	Freq.			Number	Freq.	Number	Freq.
1	40/13	0		1	0.0057	31	49/15	24	0.0603	12	0.0682
2	40/15	0		1	0.0057	32	49/16	1	0.0025	3	0.0170
3	41/14	1	0.0025	1	0.0057	33	49/17	0		1	0.0057
4	41/15	1	0.0025	0		34	50/14	8	0.0201	3	0.0170
5	41/16	0		1	0.0057	35	50/15	29	0.0729	8	0.0455
6	42/14	0		1	0.0057	36	50/16	3	0.0075	0	
7	42/15	0		1	0.0057	37	50/17	0		1	0.0057
8	43/14	1	0.0025	3	0.0170	38	51/14	16	0.0402	5	0.0284
9	43/15	5	0.0126	2	0.0114	39	51/15	27	0.0678	12	0.0682
10	43/16	0		2	0.0114	40	51/16	1	0.0025	2	0.0114
11	44/13	1	0.0025	0		41	51/17	0		1	0.0057
12	44/14	1	0.0025	3	0.0170	42	52/14	14	0.0352	0	
13	44/15	4	0.0101	5	0.0284	43	52/15	8	0.0201	5	0.0284
14	44/16	0		1	0.0057	44	52/16	1	0.0025	0	
15	45/13	0		1	0.0057	45	53/13	1	0.0025	0	
16	45/14	6	0.0151	5	0.0284	46	53/14	6	0.0151	3	0.0170
17	45/15	16	0.0402	9	0.0511	47	53/15	9	0.0226	5	0.0284
18	45/16	1	0.0025	3	0.0170	48	53/16	1	0.0025	0	
19	46/13	0		1	0.0057	49	54/14	5	0.0126	2	0.0114
20	46/14	18	0.0452	5	0.0284	50	54/15	11	0.0276	5	0.0284
21	46/15	28	0.0704	8	0.0455	51	55/13	0		1	0.0057
22	47/13	0		1	0.0057	52	55/14	4	0.0101	0	
23	47/14	13	0.0327	6	0.0341	53	55/15	3	0.0075	2	0.0114
24	47/15	32	0.0804	7	0.0398	54	55/16	0		1	0.0057
25	47/16	5	0.0126	4	0.0227	55	56/14	1	0.0025	0	
26	48/14	27	0.0678	5	0.0284	56	56/15	1	0.0025	0	
27	48/15	30	0.0754	19	0.1080	57	57/14	1	0.0025	0	
28	48/16	1	0.0025	2	0.0114	58	58/14	1	0.0025	0	
29	49/13	0		1	0.0057	59	58/15	1	0.0025	0	
30	49/14	26	0.0653	5	0.0284	60	59/14	4	0.0101	0	
Haplotype diversity									0.9519		0.9645

exists, haplotype frequencies have to be estimated directly from appropriate population sample. The haplotype frequencies of DXS6807–DXS8378–DXS9902, DXS6804–GATA172D05 and DXS8377–DXS7423, which were significant LDE in Han population, were listed in Tables 2, 3 and 4. However, no evidence for LDE was found in the Kazakh population. It is possible that this association were the result of the sample size. Linkage and LDE may impact the weight of evidence given by a DNA analysis [17, 18]. When calculating likelihood ratio in relationship testing using this 12 X-STR system, both linkage and LDE should be taken into account as described by Tillmar et al. [19].

As compared with allele frequency distribution between the targeted population and other published populations originating from Uigur [10], Mogol [10] and Sichuan Han [20] from China, Taiwanese [1], Japanese [21], Pakistani [2], Northern Italy [3], Algerian [4], Ghana [5], Africa Morocco and Madagascar [22], and Ivory Coast [23], the allele frequency distribution for most X-STR loci is different in different populations (Table S4). These results indicated that it is important and necessary to develop data bank of different ethnic groups for forensic analysis.

In conclusion, our study has demonstrated the possibility of simultaneous genotyping the 12 X-STR loci in a single reaction. Our results indicated that this multiplex system is very useful for identification analysis, and that the genetic information about the 12 X-STR loci is necessary for forensic application in two population groups from China.

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