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ORIGINAL ARTICLE

Development of the nine X-STR loci typing system and genetic analysis in three nationality populations from China

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Abstract This study is to develop a new multiplex polymerase chain reaction (PCR) system that simultaneously amplifies the nine X-chromosome short tandem repeats loci in the same PCR reaction, and to explore their polymorphism and mutation rate among three nationality populations from China. These loci included DXS6854, DXS9902, DXS6809, GATA172D05, HPRTB, DXS7423, DXS6807, DXS8378, and DXS8377. The samples of 890 (484 males and 406 females) unrelated individuals from Guangdong Han population, Xinjiang Uigur, and Inner-Mongolia Mongol were successfully analyzed using this multiplex system. The allele frequencies and mutation rates of the nine loci were investigated, and the comparison of allele frequency distribution among different populations was performed. There were 87 alleles for all the loci, and six to 18 alleles for each locus observed by our new multiplex PCR system. Polymorphism information content was 0.4998–0.9101, and power of discrimination in females was 0.6518–0.9846. Five cases with mutation of above loci were detected in 5,310 meioses. Pair-wise comparisons of allele frequencies distribution showed significant differences for most loci among different populations. Our results indicate that this multiplex system is very useful for identification analysis, and that the information about polymorphism and mutation rate is necessary for forensic application in three nationality populations from China.

Keywords X-STR · Multiplex PCR · Population data

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Introduction

It is well known that the analysis of X-chromosome short tandem repeats (X-STR) is useful for complex kinship cases such as deficiency paternity cases and relationships between putative half-sisters sibling status having the same biological father or paternal grandmother and granddaughter [1]. Recently, more and more X-STR loci have been reported for forensic purpose [2–4]. Some genetic polymorphisms of them have been conducted in a number of populations [5–7]. Although China is the largest multi-ethnic country in the world with a population of about 1.4 billion, there are few reports about both polymorphism and mutation rates of X-STR loci in Chinese populations. Therefore, further studies are requisite to evaluate the distribution of allele frequencies and to establish a database of mutation rates of X-STR loci in these populations for forensic practice. In this study, we firstly developed a new multiplex polymerase chain reaction (PCR) system to analyze simultaneously the nine X-STR markers including DXS6854, DXS9902, DXS6809, GATA172D05, HPRTB, DXS7423, DXS6807,

DXS8378, and DXS8377, and then investigated allele frequencies and mutation rates of the above loci in three nationality populations living in Mainland China.

Materials and methods

Sample preparation and DNA extraction

Blood samples were collected from 890 unrelated individuals from three nationality populations in Mainland China. In detail, there were 446 Guangdong Han individuals (279 males and 167 females), 235 Xinjiang Uigur (98 males and 137 females), 209 Inner-Mongolia Mongol (107 males and 102 females), and there are 210 family trios (father–mother–daughter) and 170 family duos (mother–son). Parents of the trios and mothers of the duos were included in the unrelated individuals. Informed consent was obtained from all the subjects after they had been given an explanation of the study. In addition, the samples were anonymous before the STR typing was started. Genomic DNA was extracted using Chelex-100 methods [8].

PCR amplification

Amplification was carried out in a 25 μl PCR reaction volume containing 1–5 ng DNA, 200 μM for each dNTP, 1× GeneAmp PCR Buffer II, 1.5 mM $MgCl_2$, 1.0 U AmpliTaq Gold™ DNA polymerase (ABI, Foster City, CA, USA). The primer concentration and detail information was presented in Table 1. Samples were amplified in Gene Amp 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 45 s, 60°C for 45 s, 72°C for 45 s, and additional 45 min at 60°C.

Sample electrophoresis and data analysis

PCR products were resolved and detected by capillary electrophoresis using ABI PRISM 3100 Genetic Analyzer with denaturing polymer 3100 POP-4™ (Applied Biosystems, Foster city, CA, USA). Fragment sizing was supported using the Genescan™-500 LIZ™ size standards. Genotype of STR was typed using GeneMapper ID ver3.1 software. Allele typing was based on home-made allelic ladders, and the K562 and 9947A (Promega Corporation, Madison, WI, USA) cell lines DNA were typed for calibrating allelic ladder.

Sensitivity testing

Sensitivity was analyzed for the amplification of the nine X-STR loci system. K562 cell DNA was diluted with quantities of 8, 4, 2, 1, 0.5, 0.25, and 0.125 ng, and each level of DNA was amplified with the multiplex system, respectively.

Table 1 Information of primer of the nine X-STR loci

Marker	UniSTS id[a]	Chromosomal. location[a]	Physical location[a]	Primer sequences (5′→3′)[a]	Amount (μM)	Dye
DXS6807	44289	Xp22.32	4743347	GAGCAATGATCTCATTTGCA AAGTAAACATGTATAGGAAAAAGCT	0.32	HEX
DXS8378	24155	Xp22.31	9370150	TTAGGCAACCCGGTGGTCC ACAAGAACGAAACTCCAACTC	0.24	ROX
DXS9902	73076	Xp22.2	15323462	TGGAGTCTCTGGGTGAAGAG CAGGAGTATGGGATCACCAG	0.4	FAM
DXS6809	69546	Xq21.33	94938089	TGAACCTTCCTAGCTCAGGA TCTGGAGAATCCAATTTTGC	1.6	FAM
GATA172D05	498601	Xq23	113174984	TAGTGGTGATGGTTGCACAG ATAATTGAAAGCCCGGATTC	0.48	HEX
DXS6854	150270	Xq25	128688898	AGCACTTCTCCTACAACCCTC CAGCCTGGGCAGTAGAGACT	0.16	FAM
HPRTB	155241	Xq26.3	133615484	TCTCTATTTCCATCTCTGTCTCC TCACCCCTGTCTATGGTCTCG	0.24	HEX
DXS8377	154756	Xq28	149566471	CACTTCATGGCTTACCACAG GACCTTTGGAAAGCTAGTGT	0.48	ROX
DXS7423	99583	Xq28	149710903	GTCTTCCTGTCATCTCCCAAC GACCTTGGCCTTTGTCTCC	0.24	HEX

[a] Data obtained from Human Genome Browser (http://www.genome.ucsc.edu/cgi-bin/hgGateway). Physical location is distance from Xp-tel in base pairs

Sequence analysis

PCR products were purified or cloned with the TOP10F Cloning Kit following the manufacturer's instructions. Then purified PCR products or the chosen clones were 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 ver 3.5 [9] was used to perform the following statistical analysis including allelic frequencies and haplotype frequencies, exact test for HWE for female data, linkage disequilibrium test between all pairs of markers. The exact test differentiation of allele frequency distribution among different populations was performed with SPSS Statistics for Windows v16.0. Polymorphism information content (PIC) was estimated according to Botstein et al. [10]. The power of discrimination in females (PD_F) and males, mean exclusion chance were calculated according to Desmarais et al. [11].

Results

The nine markers were amplified with satisfactory results (Fig. 1). DNA of 8, 4, 2, 1, 0.5, and 0.25 ng was successfully analyzed, respectively, using this multiplex system. In forensic practice, about 1–5 ng DNA is routinely used for typing, although 0.25 ng DNA is enough. Stutter bands of $n-1$ repeat units could be detected, but they are usually seldom (<5% of the area of the true peak). Repeated analysis of random DNA samples yielded consistent results. The results of K562 and 9947A control DNA were in agreement with those reported by Szibor et al. [12]. Sequences analysis of DXS6854 revealed a simple repeat motif (ATTT)n, and no intermediate alleles were found.

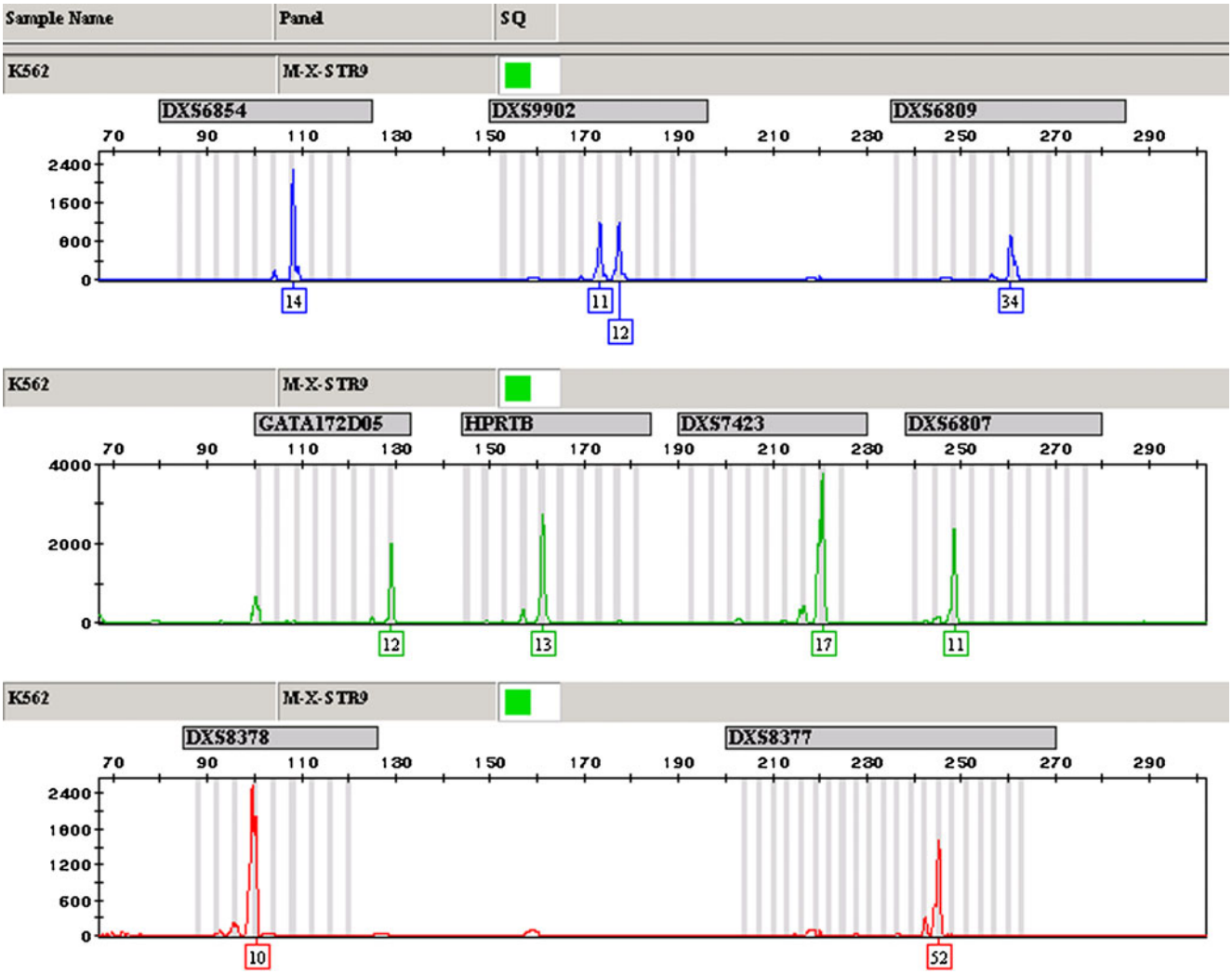

Fig. 1 The electrophoretogram of the nine X-STR loci typing system

Table 2 Allele frequencies and statistical parameter of the nine X-STR loci from the three nationality populations in China

Allele	DXS6854			DXS9902			DXS6807			DXS8378			DXS6809		
	Han	Uigur	Mongol	Han	Uigur	Mongol	Han	Uigur	Mongol	Han	Uigur	Mongol	Han	Uigur	Mongol
6	0.0065														
7		0.0027		0.0033	0.0108	0.0032									
8	0.0049	0.0054		0.0016											
9	0.2871	0.1452	0.1833	0.0147	0.0430	0.0129	0.0016			0.0196	0.0323	0.0096			
10	0.3866	0.2930	0.4116	0.4339	0.3387	0.4469				0.5285	0.4086	0.5016			
11	0.1256	0.0860	0.1125	0.3263	0.3763	0.3151	0.4829	0.4597	0.3698	0.3002	0.3387	0.2540			
12	0.1289	0.2796	0.2026	0.2104	0.2124	0.2090	0.0212	0.0457	0.0225	0.1338	0.1747	0.2315			
13	0.0506	0.1559	0.0579	0.0098	0.0188	0.0064	0.0212	0.0376	0.0289	0.0163	0.0403	0.0032			
14	0.0098	0.0296	0.0129			0.0032	0.3181	0.2688	0.3376	0.0016	0.0054				
15		0.0027				0.0032	0.1240	0.1613	0.2154						
16			0.0193				0.0277	0.0215	0.0257						
17							0.0033	0.0054							
24													0.0016		
27															0.0064
28													0.0065	0.0027	
29													0.0114	0.0134	0.0064
30													0.0408	0.0484	0.0386
31													0.1533	0.1882	0.1479
32													0.1599	0.1505	0.2090
33													0.2349	0.3360	0.2154
34													0.2055	0.1613	0.2412
35													0.1175	0.0618	0.0932
36													0.0555	0.0296	0.0257
37													0.0114	0.0027	0.0129
38													0.0016	0.0054	0.0032
K562	14			11,12			11			10			34		
9947A	13,14			11			12,14			10,11			31,34		
PD_M	0.7354	0.7776	0.6551	0.6612	0.6678	0.6336	0.6292	0.6624	0.6856	0.6108	0.6860	0.6256	0.8177	0.7897	0.8210
PD_F	0.8846	0.9206	0.9139	0.8178	0.8562	0.8247	0.8257	0.8611	0.8588	0.7861	0.8385	0.7965	0.9564	0.9311	0.9393
MECI	0.6914	0.7495	0.7032	0.5943	0.6392	0.5911	0.5900	0.6387	0.6452	0.5479	0.6279	0.5637	0.8135	0.7693	0.7939
MECII	0.5517	0.6191	0.5647	0.4471	0.4943	0.4438	0.4439	0.4938	0.5008	0.4011	0.4827	0.4153	0.7005	0.6441	0.6748
PIC	0.7330	0.7823	0.7394	0.6607	0.6962	0.6570	0.6486	0.6864	0.7009	0.6120	0.6851	0.6302	0.8346	0.7958	0.8188

Allele	DXS8377			GATA172D05			HPRTB			DXS7423		
	Han	Uigur	Mongol	Han	Uigur	Mongol	Han	Uigur	Mongol	Han	Uigur	Mongol
6				0.0522	0.1048	0.0257						
7				0.0049	0.0054	0.0064						
8				0.1762	0.1075	0.1640			0.0032			
9				0.1305	0.0565	0.0579						
10				0.3703	0.3978	0.5016	0.0065	0.0027	0.0064			
11				0.2219	0.2500	0.1897	0.0897	0.0672	0.0514			
12				0.0440	0.0780	0.0547	0.2773	0.2849	0.2508	0.0016		
13							0.4356	0.3763	0.4373	0.0049	0.0296	0.0032
13.2							0.0016					
14							0.1485	0.1935	0.1672	0.3540	0.2984	0.2765
15							0.0375	0.0699	0.0804	0.6117	0.5323	0.6431
16							0.0033	0.0054	0.0032	0.0261	0.1210	0.0740
17										0.0016	0.0188	0.0032
41	0.0049	0.0027										
42	0.0114	0.0134	0.0611									
43	0.0114	0.0215	0.0129									
44	0.0408	0.0484	0.0386									
45	0.0636	0.0753	0.0997									
46	0.0897	0.1102	0.0707									
47	0.0979	0.0887	0.1286									
48	0.1223	0.1183	0.1222									
49	0.1028	0.1398	0.1608									
50	0.1272	0.0968	0.1061									
51	0.1223	0.1022	0.0997									
52	0.0848	0.0618	0.0257									
53	0.0277	0.0430	0.0354									
54	0.0604	0.0430	0.0161									
55	0.0196	0.0081	0.0129									
56	0.0065	0.0161										
57	0.0033	0.0027										
58	0.0033	0.0081	0.0096									
K562	52		0.0096	12			13			17		
9947A	45,47			10			14			14,15		
PD_M	0.9060	0.9020	0.8853	0.7679	0.7730	0.6128	0.7069	0.7320	0.7105	0.5080	0.5786	0.5087
PD_F	0.9837	0.9846	0.9814	0.9044	0.8931	0.8751	0.8600	0.8751	0.8710	0.6518	0.7991	0.6671
MECI	0.9002	0.9031	0.8900	0.7262	0.7128	0.6404	0.6536	0.6856	0.6653	0.4053	0.5495	0.4359
MECII	0.8249	0.8296	0.8095	0.5914	0.5764	0.4948	0.5097	0.5449	0.5222	0.2729	0.4028	0.2965
PIC	0.9076	0.9101	0.8987	0.7609	0.7474	0.6785	0.7004	0.7303	0.7087	0.4998	0.6118	0.5045

PD_M power of discrimination in males, *PD_F* power of discrimination in females, *MECI* mean exclusion chance for X-STR in standard trios with daughters. *MECII* mean exclusion chance for X-STR in father/daughter duos. *PIC* polymorphism information content.

Genotype of K562 DNA and 9947A DNA were 14 and [13, 14], respectively, which have not been reported previously. A total of 80 alleles, ranging from six to 18 for each locus, were observed in 446 Guangdong Han individuals. Seventy-six alleles, ranging from five to 18 for each locus, were observed in 235 Xinjiang Uigur. Seventy-two alleles, ranging from five to 15 for each locus, were observed in 209 Inner-Mongolia Mongol. Table 2 showed the allele frequencies and further statistical information of the nine loci in the three nationality populations. Five cases of mutation, three at the DXS8377, one at HPRTB, and one at DXS6809 locus, were found through pedigree analysis of 210 father–daughter–mother trios (420 meioses) and 170 mother–son duos (170 meioses). There was not any mutation found at other six loci. The information about mutation was listed in Table 3.

Discussion

This paper presents a convenient procedure for amplifying the nine X-STR loci in a single reaction, in that PCR amplification and allele typing were successful for all these loci.

Allele nomenclature used for cell line DNA sample genotyping for most loci was done according to Szibor et al. [12]. Our sequencing data is in agreement with results of cell line DNA samples except for HPRTB locus. Sequencing analysis of our ladder samples displays that allele (13) has 14-repeat TCTA instead of 13 repeats for HPRTB locus. Its sequence is P_F-N_{23}-TCTA-TC-$(TCTA)_{14}$-N_{34}-P_R, which is in agreement with the results reported by Gomes et al. [13]. In addition, an intermediate allele (13.2) was found also in Guangdong Han population. After sequencing analysis, we found the allele (13.2) has 16-repeat TCTA due to "TC" deletion upstream from repeat region, which sequences is P_F-N_{23}-TCTA▲T▲C $(TCTA)_{15}$-N_{34}-P_R. Likewise, intermediate allele (12.2) had been reported by Mertens [14] and Gomes et al. [13]. As a matter of fact, their different results came from a deletion of a dinucleotide AG in 50 nucleotides downstream from repeat region for the 12.2. Therefore, according to Szibor's statement [15], HPRTB allelic ladders should be calibrated with cell line DNA genotypes as described to avoid unnecessary confusion. HPRTB allele ladder was calibrated also with cell line DNA K562 and 9947A genotype in our multiplex system. Our genotypes of HPRTB ladder were also in accordance with that of Argus X-8® kits and Argus X-12 kits (Biotype AG, Dresden, Germany).

Hardy–Weinberg equilibrium (HWE) was performed on female samples, and the genotype distributions did not deviate from HWE at the nine loci. Allele frequencies between female and male samples were not significantly different in all the loci. PIC of all the loci reached above 0.61 with exception of DXS7423. Especially, PIC of DXS8377 loci went beyond 0.90. PD_F of the loci went beyond 0.98. DXS8377 loci was the highest polymorphic, with the highest power of discrimination and probability of paternity exclusion among the loci studied. These results suggest that nine X-STR loci system has satisfactory forensic efficiency.

The comparison of allele frequency distribution were preformed between the targeted populations and other published populations originating from Sichuan Han in China [16], Taiwanese [17], Northern Italy [4], German [18], Japanese [19], Spanish [20], Santander [6], Portugal [21], Pakistani [22], Algerian[5], Ghana[7]. There are significant differences of most loci between Han and Uigur except at the HPRTB and DXS8377, and significant differences between Han and Mongol except at the DXS9902, HPRTB, and DXS6809, and significant differences of all the nine loci between Han and other nine reported populations (seen as Electronic supplementary Table S1) except at HPRTB in Pakistanis. However, there is not any significant difference between Guandong Han and Sichuan Han, as well as between Guandong Han and Taiwanese except at DXS7423 and DXS8377. There may be possibility that most of Taiwanese come from Han

Table 3 Mutation detected from the pedigree analysis of father–daughter–mother trios and mother–son duos

Locus	Genotype			Transmission	Age	Mutation rate (%)
	Paternal	Maternal	Child[a]			
DXS6809	32	31–36	31–*33*	Father-to-daughter	Father (32); mother (24)	0.21
DXS6809[b]	32	33–33	32–*34*	Mother-to-daughter	Father (43); mother (33)	
DXS6809[b]		32–33	*34*	Mother-to-son	Mother (23)	
HPRTB	14	12–13	12–*15*	Father-to-daughter	Father (33); mother (32)	0.17
DXS8377	45	47–47	*46*–47	Father-to-daughter	Father (30); mother (25)	0.51
		46–52	*47*	Mother-to-son	Mother (27)	
		47–51	*46*	Mother-to-son	Mother (33)	

[a] In the genotypes of children, alleles with the mutation were denoted in italics

[b] Mutations were detected by using the five X-STR loci systems [2]

population living in Mainland China. There are significant differences between Uigur and Mongol except at the HPRT, and there are significant differences between Uigur and the other selected foreign population as well. In addition, significant differences are found between Mongol and the selected populations, too. Heterogeneous marriage is not common and homogeneous marriage is prevalent because of different nationality origin, different language and culture etc. Han nationality is the largest ethnic group in China and the people are all over the place. So Han nationality marriage is random. These may possibly explain why there is no significant difference among Han in different regions but there is significant difference among other ethnic groups. The results indicated that it is important and necessary to develop data bank of different ethnic groups for forensic analysis.

The exact test for linkage disequilibrium was performed for all pairs of loci in the three nationality populations from China. The five significant results from 36 pair-wise comparisons were found between DXS6854 and DXS6807, DXS6854 and DXS8377, DXS6809 and DXS8377, DXS9902 and DXS8378, and DXS7423 and DXS8377. The highest association ($P<0.00001$) was found between DXS9902 and DXS8378, and DXS7423 and DXS8377, which have also been reported by Bekada et al. [5]. In addition, alleles of linked loci form haplotype that recombine during meioses. When linkage disequilibrium exists, haplotype frequencies have to be estimated directly from appropriate population sample [1]. In this study, our new multiplex system could develop haplotype of DXS8378–DXS9902 in Xp22 and DXS8377–DXS7423 in Xq28. Haplotype frequencies of DXS8378–DXS9902 and DXS8377–DXS7423 were listed in Electronic supplementary Tables S2 and S3. The loci distance is quite long on the chromosomes between DXS6854 and DXS6807, DXS6854 and DXS8377, and DXS6809 and DXS8377, which are not physically closely linked with each other. No real linkage disequilibrium is expected to exist between them. So, it is possible that this association were the result of the sampling effects.

In the kinship cases, 40 three-generation families (grandmother–father–granddaughter) have been tested using nine X-STR loci system developed in this study. The grand-maternal genotypes were found to be transmitted to her granddaughters by her son. Five cases of mutation were detected from the nine loci in 5,310 meioses. There are two cases of mutation found in DXS6809 locus by the five X-STR loci multiplex system [2] in 4,295 meioses. All the mutations were the shift of one repeat unit. The average mutation rate for the nine loci was estimated to be 0.98×10^{-3} per meiosis.

In conclusion, our study has demonstrated the possibility of simultaneous genotyping the nine X-STR loci in a single reaction. Our results indicate that this multiplex system is very useful for identification analysis, and that the information about polymorphism and mutation rate is necessary for forensic application in three nationality populations from China.

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