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SHORT COMMUNICATION

Development of a five ChX STRs loci typing system

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Abstract We investigated the polymorphism of five X-chromosomal short tandem repeat markers (ChrX STRs) loci (DXS7132, DXS981, DXS6803, DXS6809, and DXS6789) and their value for forensic applications. A fluorescent multiplex polymerase chain reaction (PCR) for amplifying five ChrX STRs loci in the same PCR reaction was set up. A total of 827 unrelated individuals of the Han nationality in China were tested. The results show that the five loci in the multiplex system provide high polymorphism information for forensic identification and paternity testing, particularly for difficult paternity-deficient cases.

Keywords ChrX STRs · Fluorescent multiplex PCR · Polymorphism · Han nationality in China

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Introduction

Recently, more X-chromosomal short tandem repeat markers (ChrX STRs) have been recognized as important tools for forensic and genetic analysis [1–7]. The ChrX STRs are inherited as a single haplotype in males, and the father transmits his ChrX STRs only to his daughter(s). Thus, they should share at least one allele at every locus. The case of whether presumed half-sisters share the genetic father could be tested by analysis of X-linked markers, without the parents being tested. Moreover, the ChrX STRs were also useful to confirm paternal grandmother–granddaughter relationships because granddaughter(s) share at least one identical allele in each ChrX STR locus with their grandmother [8]. The study of X-linked markers may permit solving otherwise impossible cases [9]. ChrX STRs are potentially complementary to other genetic markers (autosomal STRs, Y-STRs, and mitochondrial DNA) [1]. To develop reliable ChrX STR multiplex systems for forensic casework and to increase the pool of relevant data for allele distribution and frequency in ChrX STR, we developed a system to amplify five ChrX STRs loci (DXS7132, DXS981, DXS6803, DXS6809, and DXS6789). In this study, we described the polymorphism degree of the five markers in a Han population, as well as Harding–Weinberg equilibrium (HWE), mutation rates and haplotype data for the closely linked STRs DXS6809 and DXS6789.

Materials and methods

Sample preparation and DNA extraction

Samples were from 827 unrelated individuals of the Chinese Han nationality (500 males and 327 females),

294 family trios in which the child was female and 271 family duos with mother and son. Parents of the trios and mothers of the duos were included in the 827 unrelated individuals. Genomic DNA was extracted using Chelex-100 methods [10].

PCR amplification

According to different sizes of amplified fragments, the primers of three X-STR loci (DXS981, DXS6803, and DXS6809) were labeled with fluorochrome 6-FAM (blue). DXS7132 and DXS6789 were labeled with HEX (green). Information of primers is shown in Table 1. Primer sequences and the genetic localizations are according to data from gene banks (http://www.genome.ucsc.edu or http://www.ncbi.nlm.nih.gove/mapview).

Amplification was carried out in a 25-µl polymerase chain reaction (PCR) volume containing 10–20-ng DNA, 200 µM for each deoxyribonucleotide triphosphate, with 1.5 mM $MgCl_2$, 1×buffer, 1.5 U Taq plus DNA polymerase. The primer was 0.54, 0.40, 0.47, 0.43, and 0.49 µM for DXS7132, DXS981, DXS6803, DXS6809, and DXS6789, respectively. Samples were amplified in the Primus Thermal Cycler (MWG-BIOTECH AG, Germany) under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s, and additional 5 min at 72°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 94404, USA) with GeneMapper ID 3.1 Analysis Software. Fragment sizing was supported using the Genescan™-500 LIZ™ size standards. The K562 and 9948 (Promega Corporation, Madison, WI, USA) cell line DNA were typed for calibrating allelic ladder. Some common alleles were also sequenced to help in allele typing. Allele typing was based on in-house allelic ladder.

Statistical analysis

Haplotype and allelic frequencies were tested using ARLEQUIN 1.1. The exact chi-square test for HWE using female data was estimated with Genetic Data Analysis program (ver1.1) [11]. The haplotype diversity was calculated by the formula $HD = 1 \times \frac{n}{n-1} - \sum_{i=1}^{n} p_i^2$, the power of discrimination in females and males and mean exclusion chance were calculated with the Desmarais formula [12]. Mutation frequency was calculated by the number of mutation event per locus per meiosis.

Results

The five markers were amplified with satisfactory results (Fig. 1). Repeated analysis of random DNA samples gave consistent results. Table 2 shows the allele frequencies of the five loci and further statistical information, as well as the results of the K562 and 9948 control DNA that can be employed for calibrating allelic ladder. Table 3 shows the haplotype frequencies of DXS6809 and DXS6789 in 500 unrelated males from Han population in China. Sixty-two different haplotypes were found. The haplotype diversity reached 0.9626. Eight cases of mutation were found from the five selected loci through pedigree analysis of 294 father–daughter–mother trios (588 meioses) and 271 mother–son duos(271 meioses), three at DXS7132, two at the DXS6803, two at DXS6809, and one at DXS6789 locus. Mutations were not found at the DXS981 locus. Mutation information is listed in Table 4.

Table 1 Information of primer of the five ChrX-STRs loci

Locus	Sequence map position (bp)	Label	Size (bp)	Primer sequence
DXS7132	64,572,061–64,572,348	HEX	276~304	F: 5′- AGCCCATTTTCATAATAAATC C-3′ R: 5′-AATCAGTGCTTTCTGTACTATTGC-3′
DXS981	68,114,084–68,114,270	FAM	178~202	F: 5′- TCAGAGGAAAAGAAGTAGACATACT -3′ R: 5′- TTCTCTCCACTTTTCAGAGTCA -3′
DXS6803	86,317,826–86,317,938	FAM	97~125	F: 5′- GAAATGTGCTTTGACAGGAA-3′ R: 5′- CAAAAAGGGACATATGCTACTT -3′
DXS6809	94,824,809–94,825,067	FAM	239~279	F: 5′- TGAACCTTCCTAGCTCAGGA -3′ R: 5′- TCTGGAGAATCCAATTTTGC -3′
DXS6789	95,336,030–95,336,211	HEX	150~194	F: 5′-GTTGTTACTTAATAAACCCTCTTT-3′ R: 5′-AAGAAGTTATTTGATGTCCTATTGT-3′

Details in bp and primer sequences come from http://www.genome.ucsc.edu or http://www.ncbi.nlm.nih.gove/mapvie

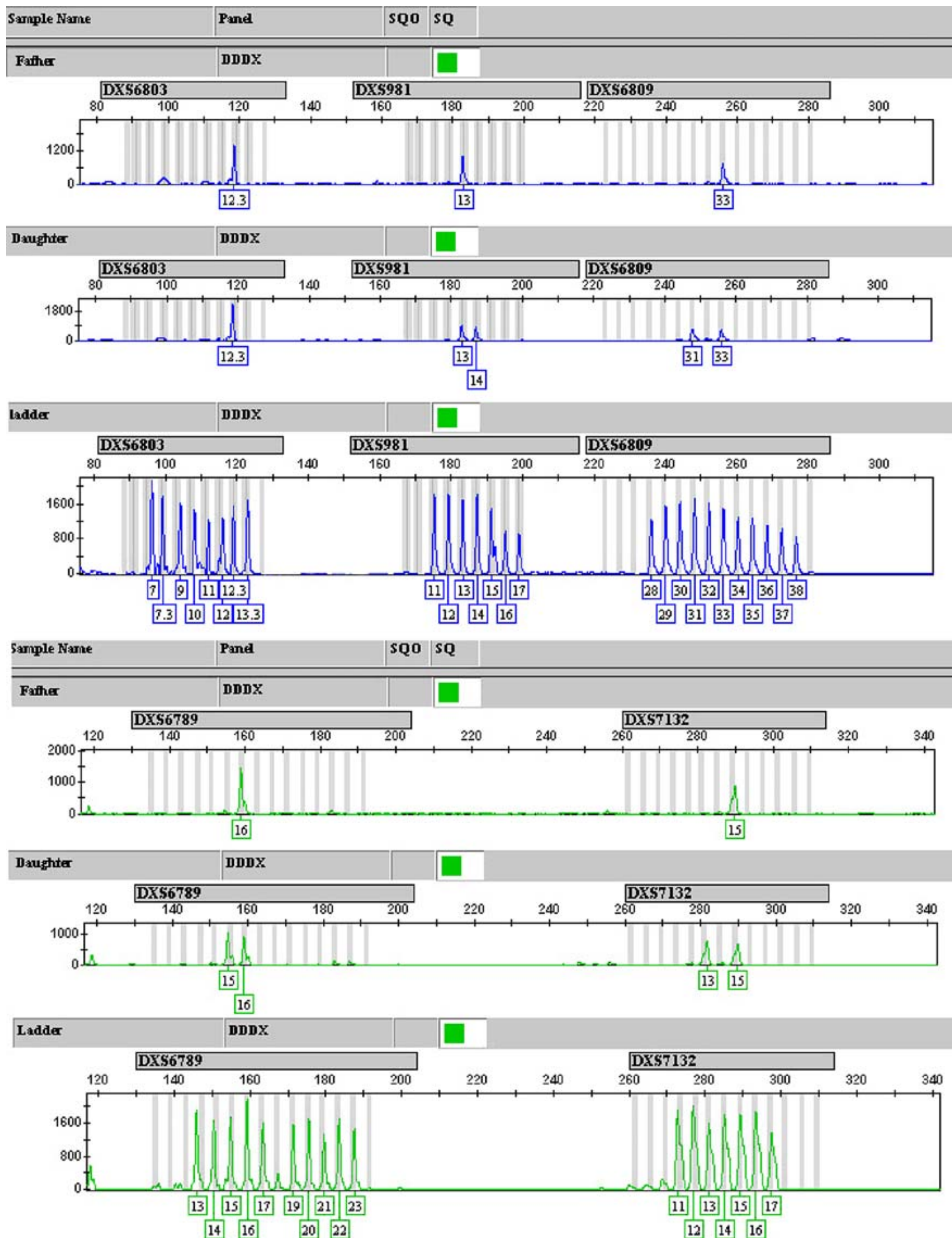

Fig. 1 The electrophoretogram of penteplex ChrX STR system

Discussion

This paper presents a convenient procedure for amplifying five ChrX STRs (DXS7132, DXS981, DXS6803, DXS6809, and DXS6789) in a single reaction. We routinely used about 20-ng DNA, although 5-ng DNA is enough for typing. Stutter bands of n-1 repeat units could be detected, but they are usually small (<5% of the area of the true peak). The results of K562 and 9948 control DNA were in agreement with those reported by Szibor et al. [13].

The five markers investigated here are located on the long arm of X-chromosome. DXS7132 and DXS981 are located at the Xq13.1, while DXS6803, DXS6809, and DXS6789 are located at the Xq21. The ChrX contains four linkage groups

Table 2 Allele frequencies of DXS6803, DXS981, DXS6809, DXS6789, and DXS7132 from Han population in China (N_{male}=500, N_{female}=327)

	DXS6803		DXS981		DXS6809		DXS6789		DXS7132	
	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.	Allele	Freq.
	7	0.0061	11	0.0095	28	0.0026	13	0.0009	11	0.0069
	7.3	0.0026	11.3	0.0017	29	0.0104	14	0.0017	12	0.0979
	9	0.0121	12	0.0485	30	0.0260	15	0.1603	13	0.1750
	9.3	0.0130	12.3	0.0815	31	0.1499	16	0.3648	14	0.3492
	10	0.1360	13	0.1508	32	0.1776	17	0.0537	15	0.2686
	10.3	0.0589	13.3	0.1872	32.1	0.0017	18	0.0009	16	0.0841
	11	0.1352	14	0.2721	33	0.2426	19	0.0121	17	0.0173
	11.3	0.4558	14.3	0.0797	34	0.2132	20	0.1993	18	0.0009
	12	0.0607	15	0.1057	35	0.1161	21	0.1326		
	12.3	0.0910	15.3	0.0295	36	0.0459	22	0.0641		
	13	0.0156	16	0.0260	37	0.0087	23	0.0078		
	13.3	0.0095	16.3	0.0009	38	0.0052	24	0.0017		
	14	0.0035	17	0.0069						
K562	9		13.3		34		21		13	
9948	12		14.3		31		20		13	
PIC	0.7163		0.8123		0.7992		0.7473		0.7004	
PD_M	0.7270		0.8405		0.8248		0.7901		0.7644	
PD_F	0.9125		0.9554		0.9460		0.9134		0.9009	
MECI	0.7153		0.8218		0.8018		0.7471		0.7210	
MECII	0.5795		0.7125		0.6850		0.6167		0.5854	
P(HWE)	0.0001		0.0039		0.6876		0.8023		0.3232	

PIC polymorphism information content, 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. *P* (HWE)*: P* values for the Hardy–Weinberg equilibrium

Table 3 Haplotype of DXS6809 and DXS6789 in males of Han nationality in China (*N*=500)

No.	Haplotype	Number	No.	Haplotype	Number	No.	Haplotype	Number
1	34/16	48	22	35/15	7	43	37/20	2
2	33/16	32	23	36/16	7	44	28/16	1
3	31/16	29	24	35/17	6	45	29/15	1
4	32/16	29	25	31/17	5	46	29/22	1
5	35/20	25	26	32/17	5	47	30/17	1
6	34/15	22	27	32/22	5	48	30/20	1
7	33/15	21	28	35/22	5	49	32/19	1
8	33/20	20	29	29/16	4	50	32.1/20	1
9	34/20	19	30	30/16	4	51	33/18	1
10	32/20	18	31	36/17	4	52	33/23	1
11	32/21	18	32	30/15	3	53	33/24	1
12	35/16	18	33	31/22	3	54	34/23	1
13	31/21	15	34	33/19	3	55	35/19	1
14	33/21	13	35	34/17	3	56	36/14	1
15	33/22	13	36	35/21	3	57	36/19	1
16	31/15	12	37	30/21	2	58	36/23	1
17	34/21	11	38	32/23	2	59	37/16	1
18	32/15	10	39	36/15	2	60	38/15	1
19	34/22	10	40	36/20	2	61	38/20	1
20	33/17	9	41	36/21	2	62	38/21	1
21	31/20	8	42	36/22	2			
Haplotype diversity								0.9626

Table 4 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]			
DXS7132	15	13–15	13–**14**	Father-to-daughter	Father (26); mother (18)	0.35
DXS7132	14	12–12	12–**13**	Father-to-daughter	Father (31); mother (26)	
DXS7132	16	14–15	14–**17**	Father-to-daughter	Father (39); mother (24)	
DXS981						0
DXS6803	12	10.3–11.3	11.3–**13**	Father-to-daughter	Father (60); mother (24)	0.23
DXS6803	13.3	11.3–12.3	11.3–**12.3**	Father-to-daughter	Father (29); mother (23)	
DXS6809	32	33–33	32–**34**	Mother-to-daughter	Father (43); mother (33)	0.23
DXS6809		32–33	**34**	Mother-to-son	Mother (23)	
DXS6789	15	16–20	**14**–20	Father-to-daughter	Father (36); mother (25)	0.12

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

[1]. At present, the five selected loci are localized to linkage group 2 [1]. As described by Szibor et al. [14], DXS6809 and DXS6789 are closely linked, so we only investigated their haplotype using male data. Sixty-two different haplotypes were found. The haplotype diversity reached 0.9626.

HWE was performed on female samples, and the genotype distributions did not deviate from HWE at the DXS6809, DXS6789, and DXS7132 loci. But genotype distributions of the DXS6803 and DXS981 deviated from HWE, as there were some rare alleles among them. When we combined these alleles, departure from HWE was not found ($P>0.05$). The results show the pentaplex ChrX STRs system had high forensic efficiency. The DXS981 locus is highly polymorph, with the highest power of discrimination and probability of paternity exclusion among the five loci studied.

Eight cases of mutation were found from the five selected loci in 4,295 meioses. All mutations were shifts of one repeat unit. The average mutation rate for the five loci was estimated to be 1.86×10^{-3} per meiosis, which is consistent with other ChrX STRs [1].

In conclusion, our study showed the pentaplex ChrX STR systems for simultaneous analysis of five loci may be used to create database of population for forensic analysis. This might provide an opportunity to develop multiplex PCR by using more loci. The five loci of the multiplex system provide high polymorphism information for forensic identification and paternity testing, particularly for difficult paternity-deficient cases.

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