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Genetic polymorphism of DNATyper X19 kit in Northern Chinese Han people

YU Zhengliang[1], CAI Huiju[1], MA Wenhua[1], MO Xiaoting[1], YAO Yiren[1], WANG Le[1], LIU Chao[2], ZHAO Xingchun[1], LI Wanshui[1,*]

[1]Institute of Forensic Science, Ministry of Public Security, Beijing 100038, China.
[2]Guangzhou Forensic Institute, Guangzhou 510000, China.

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***Corresponding author:** LI Wanshu, Institute of Forensic Science, Ministry of Public Security, Beijing 100038, China.
Email: liwanshui68@sina.com

Abstract

Objective. To evaluate the value of DNATyper X19 kit in the forensic application. **Methods.** In this study, we carried out a survey of genetic polymorphism from 374 Northern Chinese Han individuals. **Results.** A total of 140 alleles were detected among the 18 X-STR loci from 374 Northern Chinese Han individuals, 228 haplotypes were detected from the 228 males, the diversity is 100%. No markers departed from Hardy-Weinberg equilibrium after applying Bonferroni's correction for multiple testing (p=0.05/18), GATA31E08-DXS6797, DXS8377-DXS10079 were in linkage disequilibrium (LD) in this study (p=0.05/153). The polymorphism information content (PIC) was 0.434-0.908 and the discrimination power (DP) was 0.432-0.983. The combined discrimination power was 0.99999993 for male and 0.999999992 for female. The combined mean exclusion chance was 0.999993 in duo cases (CMECduo) and 0.999999994 in trio case (CMECtrio). **Conclusions.** DNATyper X19 kit showed potential value for complicated paternity cases.

Keywords

Forensic Genetics, X Chromosome, X-STR

1. Introduction

Association with sex-linked genetic characteristics has made X-STR markers good candidates for efficient complementing of autosomal and Y-chromosomal STR markers in solving kinship-deficient cases involving father-daughter, mother-son, and sister-half-sister relationships, as well as those involving segregation and incest [1-3]. In 2018, Yu et al. [4] used X-STR to solve two cases, confirming that inclusion of more X-STRs may help solve complex kinship cases, which could not be resolved via autosomal STR markers. The number of loci and linkage group are important for the application of X-STR. At present in China, Argus X-12 is the most widely used multiplex amplification system. However, this system only encloses 12 X-STR loci located in 4 linkage groups, which is compounded by the fact that polymorphism of some loci in the Chinese population is less than satisfactory, limiting the efficacy of application. Furthermore, certain X-STR loci express strong linkage disequilibrium (LD) and segregate together as haplotypes [5-8]. Therefore, to develop a multiplex amplification system, which is more suitable for Chinese population is essential. DNATyper X19 Amplification Kit is self-developed by Institute of Forensic Science, Ministry of Public Security, aiming at demand of the cases, which is suitable for Chinese people. In this study, we investigated the forensic parameters from 374 unrelated Northern

Chinese Han individuals, evaluating the value of DNATyper X19 kit in the forensic application.

2. Materials and Methods

2.1 Samples

Blood samples of 374 unrelated individuals (228 males and 146 females) were collected from North China. Blood samples were extracted using magnetic beads, and the genomic DNA was amplified with DNATyper X19 kit following manufacturer's recommendations.

2.2 The General Information of DNATyper X19 kit

The General information of all the loci in DNATyper X19 kit was shown in Table 1. 4-color fluorescent chemistry was used to enable multiplexing of 18 X-STRs and amelogenin, the amplicon size ranges no greater than 450bp, which has high efficiency and reproducibility.

Table 1. General information of all the loci in DNATyper X19 kit

Locus	Repeat motif	Chromosomal location	Allele area (bp)	Fluorescent type
Amelogenin	NA	Xp22.1-22.3	100-110	FAM
GATA31E08	$[AGAT]_n$	Xq27.1	112-162	FAM
DXS10079	$[AGAA]AGAG[AGAA]_n$	Xq12	165-220	FAM
DXS10103	$[TAGA]2CTGA[CAGA][TAGA]_n$ $[CAGA][TAGA]$	Xq26.2	260-299	FAM
DXS7132	$[TCTA]_n$	Xq12	304-351	FAM
DXS9895	$[AGAT]_nA[AGAT]_m$ $[AGAT]_nAT[AGAT]_m[AGAT]_3$	Xp22.31	370-410	FAM
DXS7133	$[ATAG]_n$	Xq23	94-145	HEX
DXS7424	$[TAA]_n$	Xq22.1	171-210	HEX
DXS7423	$[TCCA]_n$	Xq28	218-280	HEX
DXS6789	$[TATC][TATG]_m[TATC]_n$	Xq21.33	310-383	HEX
DXS9902	$[GATA]_n$	Xp22.2	134-190	TAMRA
DXS6810	$[CTGT]_1[CTAT]2[CTGT]_2$ $[CTAT]_nCAT[CTAT]_1$	Xp11.3	220-260	TAMRA
DXS8377	$[AGA]_x[GGAAGA]_y[AGA]_2$ $[GGA][AGA]_6$	Xq28	290-365	TAMRA
DXS101	$[CTT]_m[ATT]_n$	Xq22.1	375-430	TAMRA
HPRTB	$[AGAT]_n$	Xq26.2	126-178	ROX
DXS8378	$[CTAT]_n$	Xp22.31	190-222	ROX
DXS6797	$[ATCT]_n$	Xq22.3	240-285	ROX
DXS6804	$[TATC]_n$	Xq23	293-340	ROX
GATA165B12	$[AGAT]_n$	Xq24	350-390	ROX

2.3 DNA amplification

DNA amplification was conducted with an Applied Biosystems ProFlex PCR System (Life Technologies, CA, USA). The reaction volume was 10 μL, containing 5 μL of 2× PCR Master Mix, 2 μL of 5×primer set, 1μL DNA, and 2μL of ddH_2O. The amplification program, based on the manufacturer's instructions, was as follows: 95°C for 15 min, 94°C for 30 s, 59°C for 45 s, 72°C for 1 min, 28 cycles, and 72°C for 60 min.

2.4 Electrophoresis and Analysis

Electrophoresis was carried out by an Applied Biosystems 3500xl Genetic Analyzer (Life Technologies), GeneMapper ID-X v1.3 (Life Technologies) was used to analyze the DNA products. The sizes of allelic ladder and alleles were calculated via the supplied internal lane standard (Typer-500), which was modified with orange. An allele peak of 100 relative fluorescence units (RFU) was set as the threshold for peak detection.

2.5 Statistical Analysis and Quality Control

After the statistical analysis of all the genotyping, we obtained the heterozygosity (H), matching probability (MP), polymorphism information content (PIC), discrimination power (DP) and power of exclusion (PE) of the 18 X-STR loci, which were calculated by the described methods [11], combined discrimination power (CDP) and combined mean exclusion chance (CMEC) were also calculated [12-15]. All DNA polymorphism analyses were conducted according to ISFG recommendations, as described by Schneider, et al. [16].

3. Results and Discussion

Population Analysis

Full profiles were obtained from the 374 samples, the profiles of 9947A and ladder were shown in Fig.1 and Fig.2.

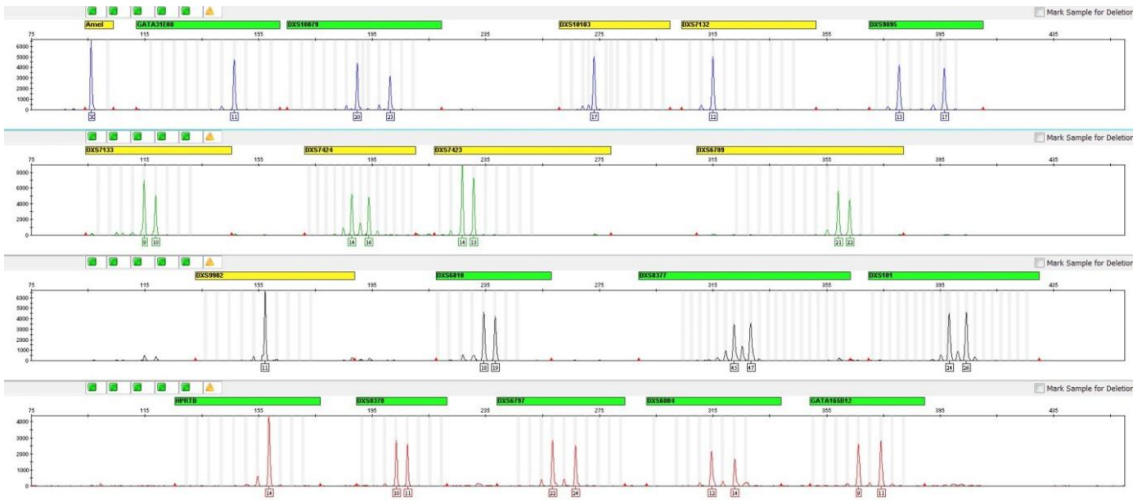

Fig. 1 Genotype profile of control 9947A at 1 ng

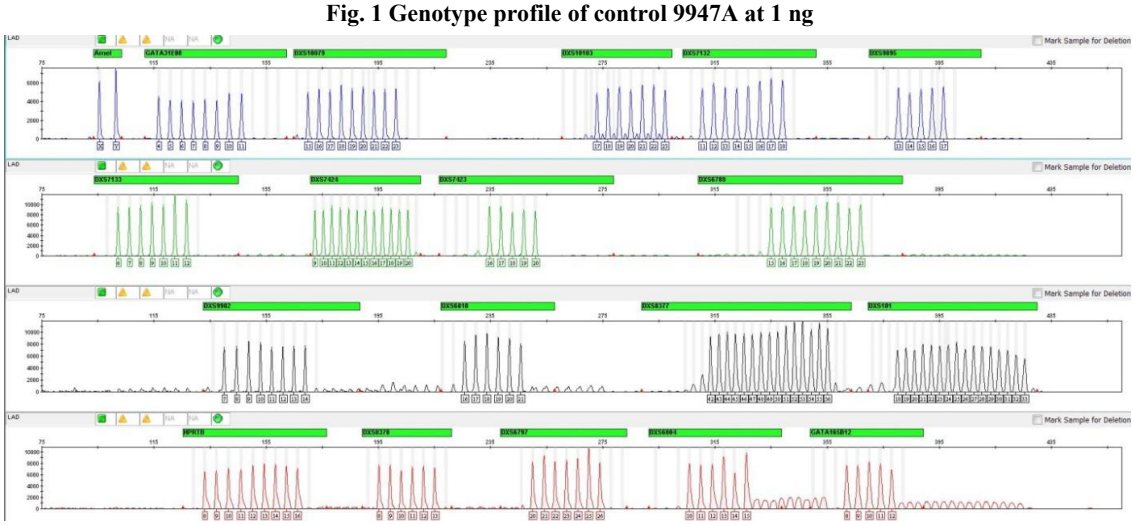

Fig. 2 Allelic ladder presenting its profile through a 3500xl genetic analyzer

The frequencies of the 18 X-STR loci were obtained from the genotyping of 374 unrelated Northern Chinese

Han individuals (228 males, 146 females). Haplotypes of the 18 X-STR were calculated by simple counting based on the 228 typed male samples, a total of 228 haplotypes having been detected, the diversity is 100%. The p-values for Hardy-Weinberg equilibrium based on 146 female samples were calculated with Powerstats software (shown in Table 2), linkage disequilibrium between 18 X-STR loci based on 228 male samples were calculated with Arlequin Ver3.5 software (shown in Table 3). No markers departed from Hardy-Weinberg equilibrium after applying Bonferroni's correction for multiple testing (p=0.05/18), GATA31E08-DXS6797, DXS8377-DXS10079 were in linkage disequilibrium (LD) in this study (p=0.05/153).

Table 2. The p-values for Hardy-Weinberg equilibrium at 18 X-STR loci (n=146)

Locus	P-values
DXS101	0.04804
DXS6789	0.96299
DXS6797	0.49029
DXS6804	0.90946
DXS6810	0.51453
DXS7132	0.64454
DXS7133	0.24846
DXS7423	0.42443
DXS7424	0.42875
DXS8377	0.13061
DXS8378	0.85857
DXS9895	0.67473
DXS9902	0.90895
DXS10079	0.13744
DXS10103	0.19116
GATA31E08	0.15229
GATA165B12	0.83427
HPRTB	0.68289

Table 3. The p-values for linkage disequilibrium between 18 X-STR loci (n=228)

	DXS101	DXS6789	DXS6797	DXS6804	DXS6810	DXS7132	DXS7133	DXS7423	DXS7424	DXS8377	DXS8378	DXS9895	DXS9902	DXS10079	DXS10103	GATA31E08	GATA165B12	HPRTB
DXS101																		
DXS6789	0.9998																	
DXS6797	0.9377	0.7254																
DXS6804	0.2594	0.5507	0.9386															
DXS6810	0.8498	0.8289	0.5212	0.4417														
DXS7132	0.8124	0.3224	0.0393	0.4267	0.6431													
DXS7133	0.2347	0.2755	0.4081	0.9279	0.7466	0.3564												
DXS7423	0.1701	0.1876	0.4627	0.1196	0.8981	0.8225	0.2412											
DXS7424	0.8721	0.1664	0.9246	0.8583	0.4799	0.9702	0.8365	0.6100										
DXS8377	0.1035	0.3967	0.8950	0.3325	0.5884	0.1519	0.9035	0.9754	0.6149									
DXS8378	1.0000	0.0608	0.9096	0.0100	0.7058	0.4910	0.0331	0.9739	0.5611	0.6527								
DXS9895	0.3619	0.2747	0.2634	0.7863	0.3454	0.4823	0.5247	0.4235	0.2064	0.3999	0.8779							
DXS9902	0.9487	0.0914	0.3254	0.8829	0.7920	0.7760	0.4098	0.1479	0.8825	0.5665	0.5857	0.7097						
DXS10079	0.3385	0.6204	0.8223	0.6169	0.8345	0.5341	0.6104	0.0654	0.2902	0.0000	0.9470	0.1076	0.8083					
DXS10103	0.0606	0.8862	0.0874	0.4245	0.1449	0.8203	0.6396	0.9854	0.9753	0.6449	0.0380	0.6596	0.1663	0.0986				
GATA31E08	0.3257	0.8908	0.0000	0.1844	0.8604	0.7470	0.5989	0.8326	0.7970	0.4248	0.9480	0.5993	0.1958	0.6734	0.0231			
GATA165B12	0.5171	0.6296	0.0821	0.7543	0.4312	0.2701	0.9251	0.6589	0.1526	0.3029	0.7605	0.5742	0.8949	0.0199	0.3544	0.5614		
HPRTB	0.6659	0.8606	0.9338	0.1062	0.9130	0.5561	0.5292	0.4444	0.8922	0.9412	0.1248	0.8383	0.8602	0.3833	0.3673	0.8206	0.1281	

A total of 140 alleles were detected from the 18 X-STR loci among the 374 individuals. There was no

significant difference in the allele frequencies between male and female populations (shown in Table 4), p=0.05. The genetic parameters of each locus (shown in Table 5) were calculated via the frequencies of the 18 X-STR loci. The combined discrimination power was 0.99999993 in male and 0.999999992 in female. The combined mean exclusion chance was 0.999993 in duo case ($CMEC_{duo}$) and 0.999999994 in trio case ($CMEC_{trio}$).

Table 4. The p-values of allele frequencies between male and female populations

Locus	P-values
GATA31E08	0.18086
DXS10079	0.68984
DXS10103	0.88359
DXS7132	0.90314
DXS9895	0.8784
DXS7133	0.61254
DXS7424	0.82461
DXS7423	0.7241
DXS6789	0.5983
DXS9902	0.92753
DXS6810	0.12524
DXS8377	0.40135
DXS101	0.25558
HPRTB	0.24508
DXS8378	0.71064
DXS6797	0.71715
DXS6804	0.85937
GATA165B12	0.97006

Table 5. Forensic parameters of 18 X-STR loci (n=374)

Locus	(H)	(MP)	(DP)		(PIC)	(PE)	
			male	female		trio case	duo case
GATA31E08	0.76	0.185	0.757161	0.905909655	0.76	0.72204	0.58695457
DXS10079	0.783	0.162	0.77980302	0.921728769	0.783	0.75002	0.62082276
DXS10103	0.764	0.177	0.76089258	0.90496499	0.764	0.72303	0.58799948
DXS7132	0.754	0.187	0.7514365	0.897400963	0.754	0.71062	0.57309016
DXS9895	0.749	0.189	0.74643626	0.89078334	0.749	0.70151	0.56195359
DXS7133	0.434	0.508	0.43264341	0.63232965	0.434	0.38687	0.25287645
DXS7424	0.728	0.212	0.7252005	0.882285889	0.728	0.683	0.54324801
DXS7423	0.534	0.384	0.53262384	0.702922892	0.534	0.45399	0.31401303
DXS6789	0.83	0.121	0.82710646	0.947427424	0.83	0.80443	0.68848312
DXS9902	0.636	0.28	0.63368013	0.792919793	0.636	0.56079	0.41478505
DXS6810	0.604	0.315	0.60186317	0.774084505	0.604	0.53446	0.38752445
DXS8377	0.908	0.063	0.90448188	0.983060916	0.908	0.89667	0.81972967
DXS101	0.818	0.139	0.81482591	0.94118208	0.818	0.7903	0.67065759
HPRTB	0.727	0.202	0.7249132	0.877935092	0.727	0.67852	0.53678585
DXS8378	0.586	0.335	0.58414226	0.76318956	0.586	0.52027	0.37440053
DXS6797	0.722	0.209	0.719265	0.874585879	0.722	0.67266	0.53051556
DXS6804	0.753	0.18	0.7499022	0.896046161	0.753	0.7085	0.57000007
GATA165B12	0.589	0.334	0.58652012	0.775672078	0.589	0.53316	0.38458674

4. Conclusion

This study demonstrated that the combined mean exclusion chance of DNATyper X19 kit is high enough for complex paternity cases, there is no significant difference compared with other systems [17-21]. Therefore, the frequencies and parameters of the 18 X-STR loci in this study can be used in the casework.

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