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Full Length Research Paper

Analysis of genetic polymorphism of nine short tandem repeat loci in Chinese Han population of Henan province

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This study was carried out to investigate the genetic polymorphism of nine short tandem repeat (STR) loci including D2S1772, D6S1043, D7S3048, D8S1132, D11S2368, D12S391, D13S325, D18S1364 and D22GATA198B05 in Chinese Han population of Henan province and to assess its value in forensic science. Genomic DNA was extracted by means of phenol-chloroform from 212 blood samples of unrelated healthy individuals in Henan Han population, and alleles were amplified with STRtyper-10F/G kit. The genotype distributions were in accordance with Hardy-Weinberg equilibrium (p>0.05). New core sequences were found in D2S1772, D7S3048, and D22GATA198B05 STR loci in Chinese Han population of Henan province, which were different from those of other populations. Two single-nucleotide polymorphism (SNP) sites were found at locus D18S1364 and D22GATA198B05, respectively. The heterozygosity (H), power of discrimination (PD), probability of exclusion (PE) and polymorphism information contents (PIC) ranged from 0.778 to 0.887, 0.928 to 0.967, 0.559 to 0.769 and 0.77 to 0.86, respectively. The total PD and combined PE of nine STR loci reached 0.999999999999363 and 0.999951, respectively. The nine STR loci are highly polymorphic in Chinese Han population of Henan province and they may be of great value in forensic science and human population genetics.

Key words: short tandem repeat, repeat motif, genetic polymorphism, Han population, forensic genetics.

INTRODUCTION

Short tandem repeat (STR) is widely used today for gene mapping, genetic linkage analysis, and human identity testing applications (Nakamura, 2009; Butler, 2007; Abrahams and Benjeddou, 2009).

In the practice of forensic science, ordinary parentage testing, individual identification cases and forensic DNA database are expected to be solved by a commercially available multiplex of STR genotyping kit, using autosomal STR kits containing 13 STR loci of combined DNA index system (CODIS), such as AmpFℓSTR Identifiler, PowerPlex™16 HS System and AmpFℓSTR Sinofiler™ kit (Butler, 2007; Ensenberger et al., 2010; Huang et al., 2010).

However, even if the three aforementioned kits were used simultaneously, the total 19 STR genetic markers were tested, which and could not provide enough discriminatory power for some difficult cases, such as complex kinship analysis, deficient cases, related individuals involved, mutations family encountered (Nothnagel et al., 2010; Phillips et al., 2008; Narkuti et al., 2010; Junge et al., 2006; Grubwieser et al., 2007). Additional STR markers are needed to complement conventional analysis

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to obtain more information; for example, some paternity testing cases containing two STR loci mutations require the testing of at least 28 STR genetic markers (Wu et al., 2010).

A new multiplex PCR system named STRtyper-10F/G kit including nine non-CODIS autosomal STR loci was reported to be highly polymorphic among the Han population in Southern China (Lu et al., 2011; Tong et al., 2010). This STR set was suitable to extend the results obtained with other STR loci commonly analyzed for difficult paternity and kinship analysis (Lu et al., 2011). However, there are still some issues to be considered, which is necessity in order to i) obtain different population genetic information from different populations; ii) know the motif of core sequence for some STR loci because there were different motif repeats reported in different populations, such as D22GATA198B05 locus (Zhao et al., 2002; Jia et al., 2005; Zhang et al., 2006) ; iii) get detailed information of different motif repeats for the compound or complex STR loci.

Due to allele structure or DNA sequence variation in the alleles of particular STR loci, sequence analysis of individual alleles is essential for the use of these markers for human identification. For the purpose of this study, we therefore analyzed the repeat motif structure of nine non-CODIS autosomal STR loci tested by means of STRtyper-10F/G kit, and investigated population genetics in Chinese Han population of Henan province to provide the basic data for forensic science and human population genetics study.

MATERIALS AND METHODS

Samples

A total of 212 unrelated individual blood samples of Han population were randomly selected in Xinxiang area of Henan province (northern of Han population) with informed consent. All blood samples collected were stored in 3.8% sodium citrate at 4℃.

DNA extraction and quantification

Genomic DNA was extracted using phenol - chloroform. The concentration of genomic DNA was measured with DNA quantitative apparatus.

PCR amplification

STR loci were amplified with fluorescent multiplex PCR system STRtyper-10F/G kit (Condon, ZhuHai, China), which comprised nine STR loci and a sex determination gene: D2S1772, D6S1043, D7S3048, D8S1132, D11S2368, D12S391, D13S325, D18S1364, D22GATA198B05, and Amelogenin. Multiplex PCR of the STRtyper-10F/G kit was performed using 10-100 nmol of template DNA in 10 µl volume including 1× Primer Set, 1× PCR Reaction Mix, and 1 U HS-Taq DNA polymerase. PCR cycle condition was 95℃ for 5 min, followed by 28 cycles at 94℃ for 1 min, 60℃ for 1 min, 72℃ for 1 min, and final extension at 72℃ for 20 min. All amplifications were done on MJ PTC-100 thermal cycler (Bio-Rad, Hercules, CA, USA).

Genotyping

PCR products were separated and detected by capillary electrophoresis using an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) with Data Collection Software (version 2.0); alleles were analyzed with GeneMapper ID v3.2 software by comparison with kit allelic ladders using the panel and bin files supplied by the kit manufacturer. When an off-ladder peak was encountered, the sample was typed repeatedly to confirm the typing. New alleles were determined and added to the allelic bin set (Phillips et al., 1998). Control DNA from cell line 9947A (Promega, Madison, WI, USA) was genotyped as standard reference in all experiments.

DNA sequence analysis

Sequence analysis was performed in both directions using the PCR primers as sequencing primers. PCR products from homozygous and heterozygous individuals were separated by electrophoresis on 6% neutral polyacrylamide gels electrophoresis, followed by silver staining. Bands of different alleles from the gels were cut and eluted with 0.5×TE, and PCR amplification was performed again for sequencing. For each purified PCR product, direct sequencing reactions were performed using the BigDye terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) by Shanghai Branch of Invitrogen Biotechnology Co. Ltd. Alleles were assigned according to the recommendations of the DNA Commission of the International Society for Forensic Haemogenetic (Bär et al., 1997).

Data analysis

The exact test of Hardy-Weinberg equilibrium was conducted with Arlequin version 3.5 software (Computational and Molecular Population Genetics Lab, Institute of Zoology, University of Berne, Baltzerstrasse 6, 3012 Bern, Switzerland, http://www.cmpg.unibe.ch/software/arlequin3). Alleles frequency and genotype frequency, as well as forensic parameters: heterozygosity (H), power of discrimination (PD), power of exclusion (PE), and polymorphism information content (PIC), were calculated by PowerStatsV12 software (Promega Company, USA, http://www.promega.com/geneticidtools/). We compared our data with STR loci allelic frequency distribution among different populations conducted in the research reported by using SPSS13.0 software package (SPSS Inc., Chicago, IL, USA). The level of significance was $p< 0.05$ for statistical analysis.

RESULTS

Sequence motif and variations of locus D2S1772 and D22GATA198B05

Through sequence analysis, core sequences of 9 STR loci showed tetranucleotide repeats. Locus D13S325 and D6S1043 displayed simple sequence repeat. Locus D2S1772, D7S3048 and D22GATA198B05 showed complex repeats and the rest STR loci were compound repeats (Table 1).

In contrast with the sequence of locus D2S1772 in

Table 1. Core sequences and physical and genetic mapping information of 9 STR loci Chinese Han Population of Henan province.

STR locus	Repeat structure	Motif repeats sequenced	Motif sequence in GenBank	GenBank accession number	Cytogenetic	UCSC	Physical (bp)
D2S1772	Complex	Motif 1: $(GATA)_n\{(CATA)(GATA)\}_2$-(CACA)(GATA)(CATA)(GATA) Motif 2: $\{(GATA)(GACA)\}_2$-$(GATA)_n\{(CATA)(GATA)\}_6$	$(GATA)_n\{(CATA)(GATA)\}_2$-(CACA)(GATA)(CATA)(GATA)	G08107	2p14	5567	67051054-67051286
D6S1043	Simple	$(ATCT)_n$	(AGAT)n	G08539	6q15	6245	92449867-92450188
D7S3048	Complex	$(TATC)_n(TACC)_n(CACC)_n$	$(TATC)_n(TACC)_n(CACC)_n$	G10359	7q15.3	6360	21266641-21266915
D8S1132	Compound	$(TCTA)_n(TCA)(TCTA)_n$	$(TCTA)_n(TCA)(TCTA)_n$	G08685	8q23.1	14549	107328821-107329072
D11S2368	Compound	$(ATAG)_n(ACAG)_n(ATAG)$	$(ATAG)_n(ACAG)_n(ATAG)$	G08890	11q15.1	6957	19280960-19281402
D12S391	Compound	$(TCTG)_n$(TCTA)n	(TAGA)n(CAGA)n	G08921	12p13.2	7052	12449874-12450226
D13S325	Simple	$(AGAT)_n$	$(AGAT)_n$	G09015	13q11.1	7211	43173250-43173571
D18S1364	Compound	$(GATA)_n(CATA)(GATA)_n$	$(GATA)_n(CATA)(GATA)_n$	G09128	18q22.1	24186	63400053-63400355
D22GATA 198B05	Complex	$(CTCT)_{1\sim2}(ATCT)_n(ACCT)_n$	$(CTCT)(ATCT)_n(ACCT)_n$	NC_000022	22q11.1	7939	17650650-17650795

Genbank(G08107), the sequence of locus D2S1772 was characterized by a complex repeat structure consisting of (GATA), (CATA), (CACA) and (GACA) repeats (Table 1), and two kinds of sequence motif were observed for locus D2S1772. One motif repeat of small alleles from heterozygous individuals showed $(GATA)_n\{(CATA)(GATA)\}_2(CACA)(GATA)(CATA)(GATA)$ (Figure 1A), which was consistent with sequence in Genbank, and another motif repeat of larger alleles from heterozygous individuals was displayed as $\{(GATA)(GACA)\}_2(GATA)_n\{(CATA)(GATA)\}_6$ (Figure 1B).

The repeat motif at locus D22GATA198B05 was also characterized by a complex STR system. The complex repeat structure consisted of (CTCT), (ATCT) and (GATA) repeats, which corresponded with the sequence in Genbank (NC_000022). The repeat (CTCT) showed 1-2 copy numbers (Figure 2A and B). Besides, we also found two SNP sites G/A and A/C in repeats region of D18S1364 (Figure 3) and D22GATA198B05 loci (Figure 4). This G/A and A/C polymorphism had no impact on the nomenclature of the two loci.

Allelic and genotyping distribution of 9 STR loci

Polymorphism of 9 loci was detected and no significant deviations from Hardy-Weinberg equilibrium were found in Chinese Han population of Henan province. Allele 24, 18, 23/24, 18, 21, 18, 20, 13 and 20 of nine STR loci was the most frequent, with a frequency of 0.285, 0.182, 0.186, 0.184, 0.255, 0.222, 0.302, 0.241 and 0.307, respectively. Total 58, 56, 51, 34, 46, 28, 33 and 41 genotypes of 9 STR loci were observed, respectively.

Forensic parameters of 9 STR loci

The H, PD, PE and PIC ranged from 0.778 to 0.887, 0.928 to 0.967, 0.559 to 0.769 and 0.77 to 0.86, respectively (Table 2). Thus, the mean values of H, PD, PE and PIC were 0.832, 0.955, 0.661, and 0.830 of nine STR loci, respectively. The nine STR loci were located at different chromosomes and the loci were independent, the total PD and combined PE reached 0.999999999999363 and 0.999951, respectively.

DISCUSSION

STR genetic markers are highly variable, and

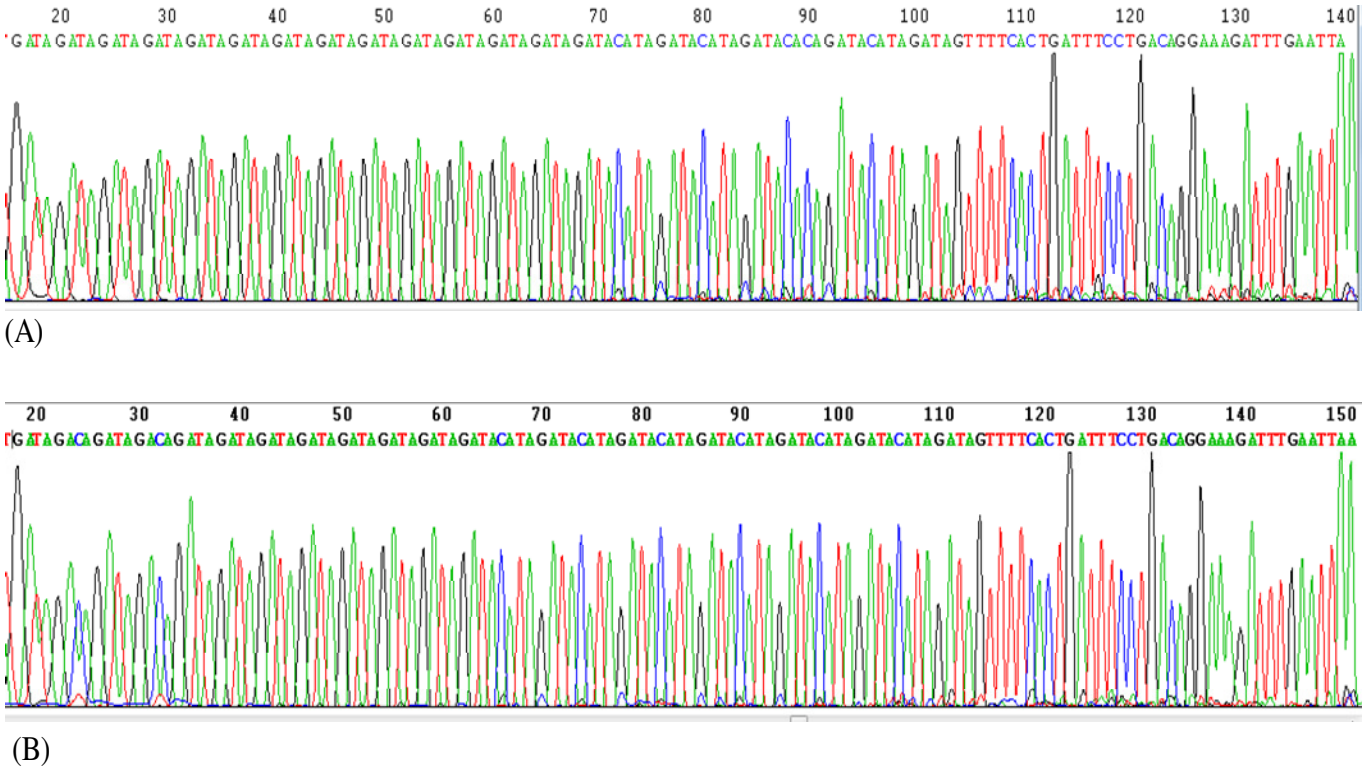

Figure 1. (A) One motif of D2S1772 locus: $(GATA)_{14}\{(CATA)(GATA)\}_2(CACA)(GATA)(CATA)(GATA)$; (B) another motif of D2S1772 locus: $\{(GATA)(GACA)\}_2(GATA)_8\{(CATA)(GATA)\}_6$.

therefore routinely used in forensic investigations for a DNA-based individual identification. The STRtyper-10F/G system is an highly polymorphic non-CODIS autosomal STR genetic markers, and researches regarding the polymorphism of these loci in southern China population have been published (Lu et al., 2011; Tong et al., 2010), but not in northern China. In this study, we reported the repeat motif of alleles and genotype distributions of 9 STR loci in Chinese Han population of Henan Province. The nine non-CODIS STR loci of STRtyper-10F/G system are shown to be more efficient and polymorphic than that of the CODIS loci system (Lu et al., 2011; Tong et al., 2010). However, there were different sequence motif repeats for some STR loci reported in different populations (Zhao et al., 2002; Jia et al., 2005; Zhang et al., 2006). From the 9 STR loci in Chinese Han population of Henan province, locus D6S1043 and D13S325 showed only a simple repeat structure, whereas compound repeat structures were present at loci D8S1132, D11S2368, D12S391, and D18S1364. Three loci of the 9 STRs showed complex repeat sequences (locui D2S1772, D7S3048 and D22GATA198B05). Only locus D2S1772 was inconsistent with the sequence in GenBank (G08107) having an impact on the nomenclature of the loci. There was no variation observed in the flanking region of 9 STR loci. Core sequence motif of locus D7S3048, D2S1772 and D22GATA198B05 differed from those of other populations. Repeat motif of D7S3048 locus showed $(TATC)_n(TACC)_n(CACC)_n$, which was different from that of motif $(GGTA)_n(ACAG)_n(ATAG)_2$ in Jiangxi Han population in China (Zhao et al., 2002). Repeat motif of D22GATA198B05 locus varies in different populations according to several published reports. Repeat motif of locus D22GATA198B05 was $(AGGT)_n(ACAG)_n(ATAG)_n$ in Jiangxi Han (Zhao et al., 2002) and $(ATCT)_n(CTAC)_n$ in Chinese Han population (Jia et al., 2005), while in this study, $(CTCT)_{1\sim2}(ATCT)_n(ACCT)_n$ was found in Chinese Han population of Henan province. We also found two repeat patterns of D2S1772 locus including $(GATA)_n\{(CATA)(GATA)\}_2(CACA)(GATA)(CATA)(GATA$ and $\{(GATA)(GACA)\}_2(GATA)_n\{(CATA)(GATA)\}_6$, which were apparently different from (GATA)n in southern area of JiangSu province in China (Zhang et al., 2006). In addition, we also found two base variation sites at repeat regions of D18S1364 and D22GATA198B05 loci, which may be two single polymorphism (SNP) sites needed to be detected by other methods and thus further enhance the genetic polymorphism. To determine whether the difference is reflective of regional differences, we need more detailed information on different populations and more extensive research.The nine STR loci are located at different chromosomes, and these loci are more than 10 Mb apart from each other. Genetic linkage disequilibrium (LD) in the human genome is generally reported at distance of 50-100 Kb. Thus, LD between the current

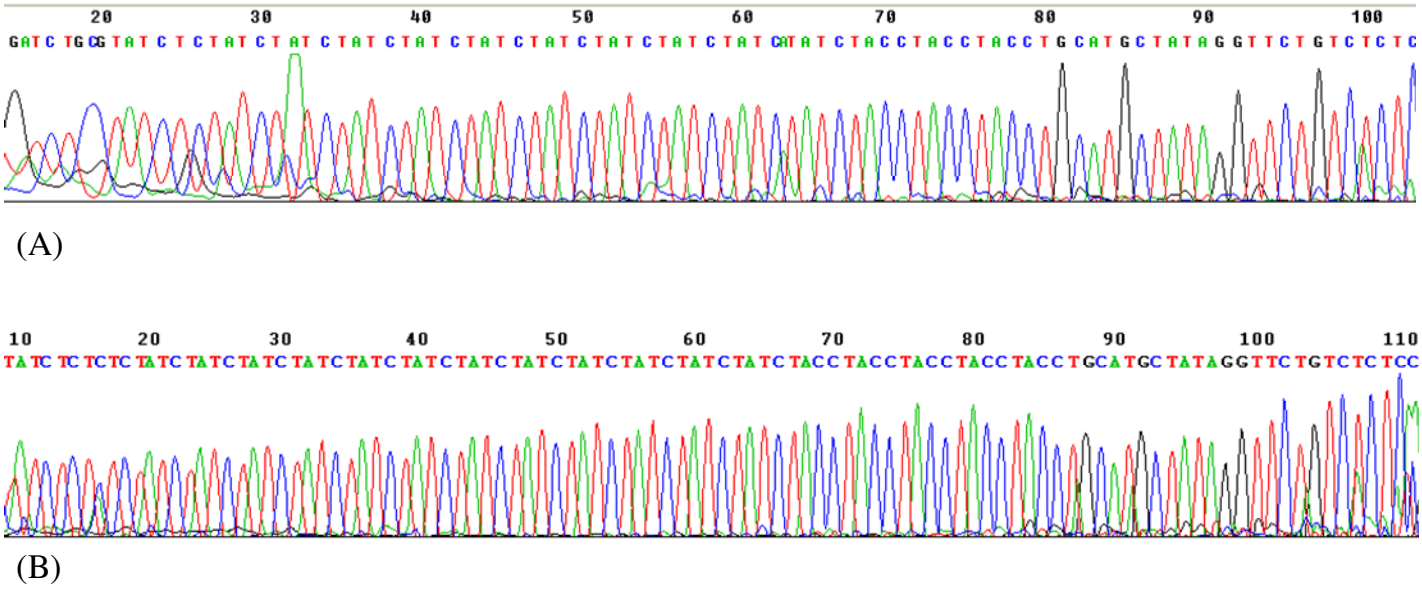

Figure 2. Complex repeats of (A) D22GATA198B05 locus—$(CTCT)_1$ $(ATCT)_{10}$ $(ACCT)_3$; (B) D22GATA198B05 locus—$(CTCT)_2$ $(ATCT)_{12}$ $(ACCT)_5$.

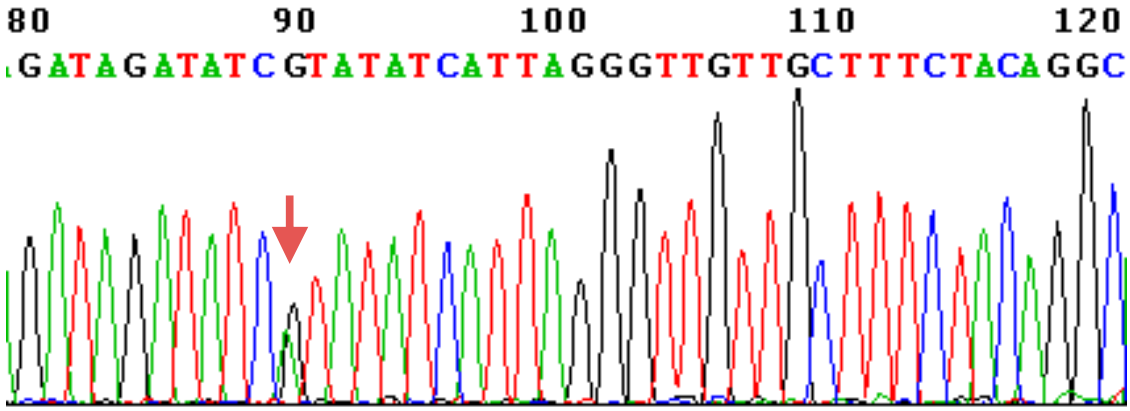

Figure 3. One SNP site (G/A) on D18S1364 locus.

non-CODIS nine loci may be excluded from consideration (Tong et al., 2010). In this study, no significant deviations from Hardy-Weinberg equilibrium were observed in any of the 9 STR loci ($p > 0.05$). A further requirement for the application of markers in forensic casework is needed for detailed population studies. It seems to be different in different groups. There was no significant difference on allelic distribution of 9 STR loci between north and south China Han population ($p > 0.05$). Compared with Japanese and Poland populations (Asamura et al., 2008; Kuzniar et al., 2006), there existed some difference on D8S1132 locus ($p < 0.05$). The 9 STR loci selected in our study with $H > 0.7$, $PD > 0.9$ satisfied filter conditions for STR locus, thus they were ideal genetic markers in Chinese Han population of Henan province. The mean values of H, PD, PE and PIC were 0.832, 0.955, 0.661, and 0.830 of nine STR loci, respectively, which were consistent with South Han population in China. If the PD of one STR locus was greater than 0.80 or/and its PE was greater than 0.50, the STR locus could be considered to be a highly polymorphic locus (Shriver et al., 1995). The results of this study showed that the PD of all loci tested were greater than 0.9, and the PE were greater than 0.50. Besides, considering the loci as independent, total PD for the nine loci and combined PE reached 0.999999999999363 and 0.999951, respectively. In summary, this set of nine STR genetic markers investigated in Chinese Han population of Henan Province sequenced the allelic motif repeats and found to be highly polymorphic, which had some prospects in the practice of forensic science, and could found the basis for population genetics research.

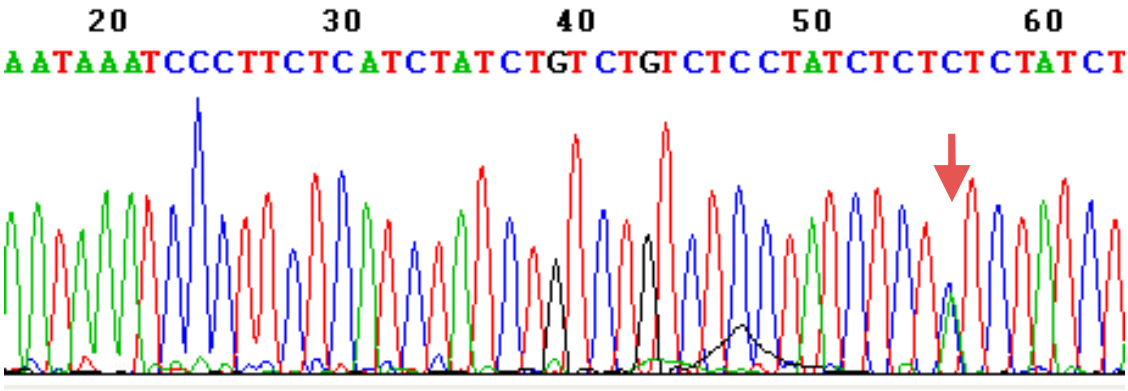

Figure 4. One SNP site (A/C) on D22GATA198B05 locus.

Table 2. Allele frequency and forensic application parameters of 9 STR loci in Chinese Han Population of Henan province.

Allele	D2S1772	D6S1043	D7S3048	D8S1132	D11S2368	D12S391	D13S325	D18S1364	D22GATA198B05
9		0.007							
10		0.024							
11		0.101						0.042	
12		0.134				0.005		0.142	
13		0.123						0.241	0.005
14		0.125				0.002		0.189	0.012
15		0.021			0.007	0.007		0.198	0.066
16			0.002	0.002	0.024	0.005		0.075	0.151
17	0.009	0.047	0.019	0.087	0.130	0.078	0.005	0.066	0.078
18	0.019	0.182	0.080	0.184	0.127	0.222	0.038	0.040	0.075
19	0.026	0.167	0.085	0.167	0.149	0.212	0.212	0.005	0.134
19.3						0.009			
20	0.068	0.038	0.146	0.177	0.139	0.160	0.302	0.002	0.307
20.3		0.002							
21	0.092	0.012	0.127	0.116	0.255	0.099	0.184		0.132
21.3		0.007							
22	0.073		0.052	0.165	0.090	0.123	0.156		0.031
22.3		0.005							
23	0.007	0.002	0.187	0.076	0.052	0.050	0.066		0.002
24	0.285	0.002	0.187	0.026	0.021	0.007	0.016		0.007
25	0.066		0.087		0.007	0.012	0.016		
26	0.078		0.024			0.007	0.005		
27	0.130		0.002			0.002			
28	0.111		0.002						
29	0.033								
30	0.002								
Ho	0.873	0.887	0.858	0.835	0.778	0.811	0.825	0.792	0.830
MP	0.038	0.033	0.035	0.042	0.043	0.044	0.072	0.047	0.054
PD	0.962	0.967	0.965	0.958	0.957	0.956	0.928	0.953	0.946
PE	0.740	0.769	0.712	0.665	0.559	0.620	0.647	0.585	0.656

Table 2. Contd.

PIC	0.85	0.86	0.85	0.83	0.83	0.83	0.77	0.81	0.81
TPI	3.93	4.42	3.53	3.03	2.26	2.65	2.86	2.41	2.94
Pvalue	0.500	0.241	0.774	0.555	0.051	0.174	0.387	0.657	0.052

H is Heterozygosity, PD is power of discrimination, PE is power of exclusion, PIC is polymorphism information content, TPI is typical paternity index.

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Abbreviations

STR, short tandem repeat; **H,** heterozygosity; **PIC,** polymorphism information content, **CODIS,** combined DNA index system; **PD,** power of discrimination; **PE,** probability of exclusion; **SNP,** single-nucleotide polymorphism; **PCR,** polymerase chain reaction; **TPI,** typical paternity index.

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