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Development of a new 17 Y-STRs system using fluorescent-labelled universal primers and its application in Shanxi population in China

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ABSTRACT

Y-chromosomal short tandem repeats (Y-STRs) polymorphisms are useful in forensic identification, population genetics and constructing of human structures. Increasing the number of Y-STRs and their polymorphism will drastically narrow down the matching number of genealogy populations or pedigrees when searching against a forensic DNA databank. In this study, we develop a system containing 17 complementary Y-STRs that are compatible and reinforce the current commercially available Y-STR kits. This system was constructed by multiplex PCR with expected size of 126bp-400bp using home-made universal primers labeled by different fluorescence (DYS715, DYS709, DYS716, DYS713, DYS607, DYS718, DYS723, DYS708, DYS714, DYS712, DYS717, DYS721, DYS605, DYS719, DYS726, DYS598 and DYS722). The genetic data were obtained from 394 individuals in Shanxi province, China. The Y-STR system has 131 haplotypes and high discrimination power is 1. In conclusion, our study provides a robust, sensitive and cost-effective genotyping method for human identification, which is beneficial for narrowing the searching scope when applying to the genealogy searching with Y-STR DNA databank.

1. Introduction

With the rapid development of DNA analysis technology, STR genotyping methods containing multiplex PCR with fluorescently labeled primers and capillary electrophoresis, have been conventionally employed in the field of forensic medicine for individual identification and paternity testing [1]. The Y-chromosome acts as a unique tool for forensic investigations since it is inherited through the patrilineal line in a relatively conserved manner. A number of commercial Y-STR kits are available and most incorporate 19–36 markers into the single multiplex systems, which have been validated for casework [2,3]. Despite the robustness of these commercial multiplex Y-STR systems and the ability to discriminate two male individuals in most cases, the coincidence match probabilities are modest compared with a set of standard autosomal STR markers. Hence, there is still a need to develop new Y-STR markers to supplement these cases where additional discriminatory power is desired or where there is a coincidental Y-STR match between potential male participants.

In this study, we developed a new 17 Y-STR typing kit, which exceeds the current Y-STR system containing the trinucleotide loci DYS718, DYS719, the tetranucleotide loci DYS715, DYS709, DYS713, DYS607, DYS708, DYS723, DYS712, DYS605, DYS726 and DYS722, the pentanucleotide loci DYS716, DYS714, DYS717, DYS721 and DYS598 [4–6]. We then developed a new kind of genotyping method using FAM/JOE/TAMRA/ROX-labeled universal M13 primers. With direct amplification and 4-dye fluorescence labeling M13 (−21) primer [7], this Y-STR kit was designed for personal identification and paternity testing.

2. Material studied

FTA blood samples were from 394 unrelated males in Taiyuan City (Shanxi Province) with the approval of the Ethics Committee of Shanxi Medical University. Written informed consent was obtained from all participants. The extraction and quantification process was performed as the manufacturer's instruction.

Sequences for each locus were obtained from NCBI using a standard nucleotide BLAST (Basic Local Alignment Search Tool) search. We selected 17 autosomal Y-STRs, which were classified into four sets (Sets A, B, C and D) based upon the needs of multiplex amplification.

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Table 1
General information on loci used in the new 17 Y-STR system.

STR locus	Repeat motif	No. alleles	Amplicon size (bp)	Primer sequences	Primer concentrations (uM)	9948	2800M
SetA							
FAM-M13				TGTAAAACGACGGCCAGT	0.25		
DYS715	(TAGA)m	7	125–149	TGTAAAACGACGGCCAGTATGGTTGGAAGAAAGCATTGATGA	0.04	14	12
				CATCCATCCATCACATCTATATCATCTTTA	0.04		
DYS709	(TTCT)4CTCT(TTCT)2(CTTTTCT)2CTT(TTCT)m	8	175–199	TGTAAAACGACGGCCAGTTCTTTCCAATGACCAAGACGTG	0.008	20	25
				TGCAAATTGTTCACATGTACCCT	0.075		
DYS716	(CACTC)m(CATTC)n	5	227–247	TGTAAAACGACGGCCAGTTAAATCAGAATTCCTTTCCAATCCA	0.04	15	16
				TCTGGGTTTCAGAGTGGGATAATT	0.1		
DYS713	(TCTT)mTC(TCTT)2(TCTG)1(TCTT)TTT(TCTT)TC (TCTT)nTT(TCTT)5CCT(TCTT)TC(TCTT)T(TCTT)	12	267–311	TGTAAAACGACGGCCAGTCCAGAAATAGATTTATTCACGCTTG	0.08	43	45
				CCTGGGTGACAGACTCCATCTTAAA	0.8		
DYS607	(AAGG)m	7	369–393	TGTAAAACGACGGCCAGTCATACAGCGTAATCACAGCTCAC	0.04	15	12
				GTAATGATGCCTCCAGTAACCAA	0.1		
SetB							
Joe-M13				TGTAAAACGACGGCCAGT	0.25		
DYS718	(TTA)m	7	153–171	TGTAAAACGACGGCCAGTGGAGAAAATTCAATGCAGTTACC	0.08	15	13
				ACACCAGCTTGGCACATTTA	0.6		
DYS723	(GATA)2TAT(GATA)mGAT(GATA)1GAT(GATA)7	8	195–223	TGTAAAACGACGGCCAGTGACAGGTGGATGCATAAATGG	0.08	19	21
				TCTGGCATCTGTCTGCATATTT	0.1		
DYS708	(GATA)m (GACA)n	8	249–277	TGTAAAACGACGGCCAGTAGTGTATCCGCCATGGTAGC	0.08	28	28
				CTGCATTTTGGTACCCCATA	0.2		
DYS714	(TTTTC)m(TCTTC)2(TTTTC)2(TCTTC)2(TTTTC)2	13	282–342	TGTAAAACGACGGCCAGTGCATCGATCTTTCTGGGAGC	0.08	32	33
				GTGTGATGCTGACTTTGGGG	0.2		
SetC							
TAMRA-M13				TGTAAAACGACGGCCAGT	0.25		
DYS712	(AGAT)m(AGAC)n	18	169–237	TGTAAAACGACGGCCAGTCAAGAACAGCCTGGGTAACAGTG	0.016	19	19
				TATATGGTACAGCCCATGAACACTT	0.1		
DYS717	(TGTAT)2TAT(TGTAT)(TGTAT)m(TGTAT)n	7	257–287	TGTAAAACGACGGCCAGTGGCCGAGAGAATGGAATTGAT	0.08	19	19
				CCCGAACTTCAGCACTATGAAATG	0.2		
DYS721	(AAGGG)mN10(AAGGG)2N7(AAGCA)6	7	298–328	TGTAAAACGACGGCCAGTGGGTGATAGAGGGAGGCTTCT	0.016	19	20
				CGGGCATGAGCTATTGAGTC	0.1		
DYS605	(TATC)4CA(TATC)3CG(TATC)m	6	380–400	TGTAAAACGACGGCCAGTAACATCTGGCTTACTTGTAGGTAG	0.08	20	18
				TCTTTGTCAGACAATGATCTGTAA	0.2		
SetD							
ROX-M13				TGTAAAACGACGGCCAGT	0.25		
DYS719	(ATA)m	7	171–189	TGTAAAACGACGGCCAGTTGACGAGTTAATGGGTGCAG	0.016	12	13
				GGAGAAAATTCAATGCAGAT	0.1		
DYS726	(CTTC)3N13 (CTTC)mN21(CTTT)3T(CTTT)2	7	214–238	TGTAAAACGACGGCCAGTGGGTAAACCTCTGAAGACCATAC	0.04	9	9
				GAATGACAGACCAAGACTCTCTC	0.1		
DYS598	(AGAAC)m	5	267–287	TGTAAAACGACGGCCAGTCTTTATTAGGCAGGCAGTTTTG	0.04	7	9
				CCAGACAATGTATGAGCAAGC	0.1		
DYS722	(GAAA)mAAGA(GAAA)2A(GAAA)2GAGA(GAAA)2	11	336–376	TGTAAAACGACGGCCAGTCCACTCATCAGTGCTCAGCTA	0.04	21	20
				GCCAACCAGCAATGTTGTC	0.1		

We used universal fluorescent PCR to detect the STRs. An 18-base universal M13 (−21) sequence (TGTAAAACGACGGCCAGT) was used as a standard sequence. The universal tail was added to the 5′ termini of the forward primers in each set. Primer-related information on each locus was listed in Table 1.

All individuals were analyzed in four independent multiplex PCRs (Sets A, B, C, and D). Each PCR was performed by using 1 ng of DNA in a total volume of 15 μL, containing (0.008 μM–0.8 μM) forward primers and reverse primers, 0.25 μM of the fluorescent universal M13 (−21) primers, and 1x PCR MasterMix (Mei5 Biotechnology (Beijing) Co, Ltd). Reaction conditions were as follows: 95 ℃ for 10 min; 25 cycles of 95 ℃ for 25 s, 56 ℃ for 25 s, and 72 ℃ for 25 s; 8 cycles of 95 ℃ for 25 s, 53 ℃ for 25 s, and 72 ℃ for 25 s; and a final extension at 72 ℃ for 60 min. The PCR products were subjected to standard capillary electrophoresis by 3130 Genetic Analyzer (Applied Biosystems).

The allele and haplotype frequencies were counted directly. Gene diversity (GD) and haplotype diversity (HD), match probability (MP) and discrimination capacity (DC) were calculated as described by Jian Zhang et al. [8].

3. Results and discussion

The representative electropherogram of a single sample is shown in Fig.1. Allele frequencies of the 17 Y-STRs in Shanxi population were listed in Table S1. The allele frequency ranged from 0.0025 to 0.7716. A total of 131 haplotypes were detected. Haplotype diversity (HD) ranged from 0.3721 (DYS598) to 0.9125 (DYS712). Match probability (MP) ranged from 0.0898 (DYS712) to 0.6288 (DYS598). The discrimination capacity (DC) was 1.

4. Conclusion

As a result of this study, we developed a new 17-plex Y-STR typing system for forensic genetic testing, which incorporated the loci out of the current Yfiler Plus kit. Allele frequencies of the 17 Y-STRs would be used for forensic personal identification and paternity testing in the Shanxi population.

Declaration of Competing Interest

None.

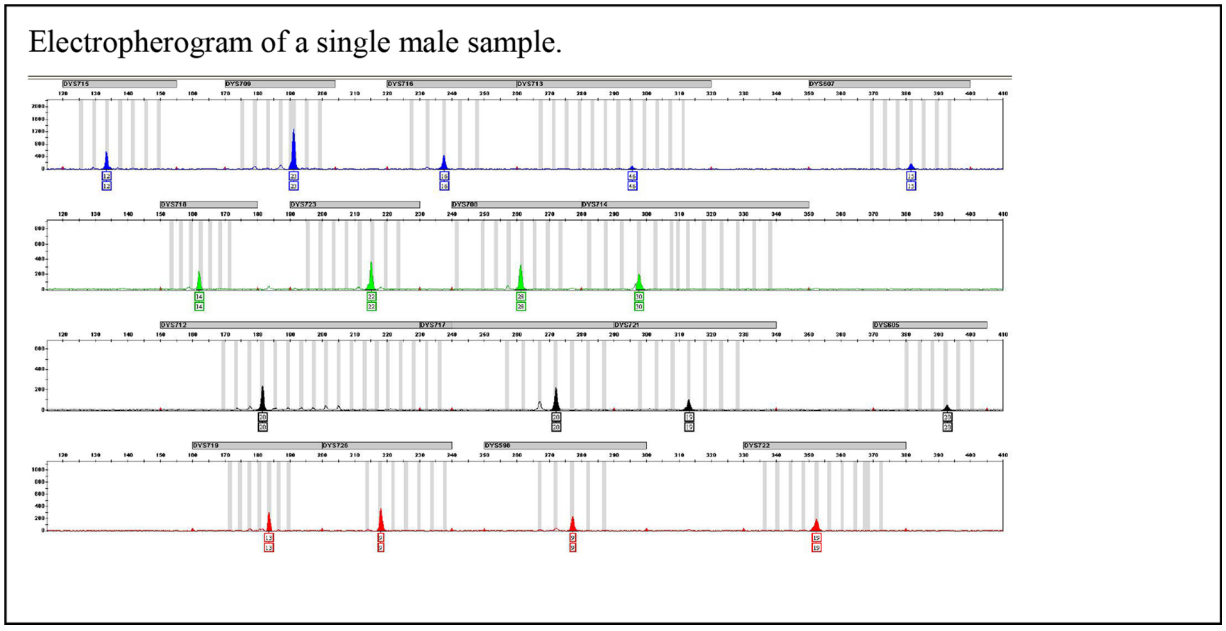

Fig. 1. Electropherogram of a single male sample.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fsigss.2019.09.037.

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