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Development of a new 26plex Y-STRs typing system for forensic application

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ABSTRACT

In this study, 26plex Y-STRs typing system, including 17 Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385ab, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATA H4) recommended as YHRD standard loci and nine new highly discriminating Y-STRs (DYS549, DYS643, DYS388, DYS570, DYS533, DYS576, DYS460, DYS481 and DYS449), was established with 5-dye fluorescences labelling. Developmental validation indicated that the 26plex Y-STRs typing system was reproducible, accurate, sensitive and robust. The sensitivity of the system was such that a full profile was obtainable even with 125 pg of male DNA. Specificity testing was demonstrated by the lack of cross-reactivity with a variety of commonly encountered animal species and bacteria. Also, the multiplex is suitable for mixture study. An average of above 97% of the minor alleles detected with the male/male mixture with 1:3 and 3:1 ratios, while an average of above 70% of the minor alleles detected with the male/male mixture with 1:19 and 19:1 ratios. Full profiles are consistently detected with 125 pg of male DNA, even in the presence of excessive amounts of female DNA. In addition, the whole PCR amplification of the 26 Y-STRs can finish in 1 h, making the multiplex system suitable for fast-detection. For the forensic evaluation of the multiplex system, 516 haplotypes were found among 517 unrelated males. HD of the multiplex system was 0.9999925 while DC was 0.9980658, which is suitable for forensic application.

1. Introduction

Y chromosome contains the largest non-recombining block in human genome and can be used to trace the male line of descent [1]. In recent years, an explosion in Y-STRs in human populations has been witnessed. This explosion has been driven, in part, by the many discovered polymorphisms of Y-STRs. By virtue of its many polymorphisms, it is now the most informative haplotyping system. Y-STRs are widely used in forensic genetics, such as mixture identification in sexual assault cases and patrilineal relationship evaluation in kinship testing [2–4]. Developing a system with highly polymorphic Y-STRs is beneficial to increase the power of discrimination among unrelated males and to reduce the chance of the existence of common haplotypes [5–7].

In this study, a new multiplex system that allows co-amplification of 26 Y-STRs, including 17 Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385ab, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATA H4) recommended as YHRD Standard loci and nine new highly polymorphism Y-STRs (DYS549, DYS643, DYS388, DYS570, DYS533, DYS576, DYS460, DYS481 and DYS449), was developed. Also, developmental validation studies were applied following the guidelines of "Validation Guidelines for DNA Analysis Methods (2012)" issued by Scientific Working Group on DNA Analysis Methods (SWGDAM) [8] and the Chinese National Standard (CNS) "Basic Quality Requirements of Forensic Science Human Fluorescent STR Multiplex PCR Testing Reagent" (GA/T815-2009). Studies of reproducibility, accuracy, species specificity, sensitivity, sizing

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precision, mixture study, PCR-based studies and population genetics were conducted. The results showed that the 26plex Y-STRs typing system with highly polymorphism in Chinese Han population is robust and reliable for forensic application.

2. Materials and methods

2.1. Loci selection

Except the 17 Y-STRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and GATA H4) which were listed as YHRD Standard loci, nine new highly discriminating Y-STR loci (DYS549, DYS643, DYS388, DYS570, DYS533, DYS576, DYS460, DYS481 and DYS449) were also chosen in the final assay (http://www.yhrd.org/), and DYS570 and DYS576 are considered as hypermutable system (http://www.yhrd.org/). Flanking regions of these Y-STRs were obtained from the University of California Santa Cruz Genome Browser (Human February 2009 Assembly; GRCh37/hg19) at http://genome.ucsc.edu/.

2.2. Primer designing and optimization

PCR primer designing of each Y-STR was performed with primer premier v5.0 and Oligo v6.0, applying the following main criteria: primer length of 15–35 bp; optimum T_m from 50 °C to 60 °C; optimum CG content range from 45% to 55%; and the amplicon length limited to 50–500 bp. A large range of the alleles included in the YHRD web (http://www.yhrd.org/) and the previous data collected in our laboratory were taken into account when designing the primers. Obtained primer pairs were then checked for non-specific hybridizations in other genome regions using NCBI Basic Local Alignment Search Tool (BLAST) at http://blast.ncbi.nlm.nih.gov/. AutoDimer v1.1 software was also applied to screen candidate primer sequences for inter-primers compatibility.

Table 1
General information of 26 Y-STRs and genotyping results of control DNA.

Y-STR	Genbank accession	Repeat Motif	Genotypes of Control DNA		
			007	XY5	9948
DYS456	AC010106	AGAT	15	14	17
DYS549	AC010133	GATA	13	12	13
DYS439	AC002992	AGAT	12	12	12
DYS19	AC017019	TAGA	15	16	14
DYS392	AC011745	TAT	13	12	13
DYS643	AC007007	CTTTT	11	12	11
GATA H4	AC011751	TAGA	13	10	12
DYS391	AC011302	TCTA	11	10	10
DYS388	AC004810	ATT	12	13	12
DYS570	AC012068	TTTC	17	19	18
DYS635	AC004772	TSTA compound	24	21	23
DYS448	AC025227	AGAGAT	19	19	19
DYS437	AC002992	TCTA	15	14	15
DYS533	AC053516	ATCT	13	12	12
DYS393	AC006152	AGAT	13	15	13
DYS389I	AC004617	(TCTG) (TCTA) (TCTG) (TCTA)	13	13	13
DYS390	AC011289	(TCTA) (TCTG)	24	23	24
DYS389II	AC004617	(TCTG) (TCTA) (TCTG) (TCTA)	29	31	31
DYS438	AC002531	TTTTC	12	10	11
DYS576	AC010104	AAAG	19	17	16
DYS460	AC009235	ATAG	11	11	11
DYS458	AC010902	GAAA	17	15	18
DYS481	BV208971	CTT	22	28	24
DYS385	AC022486	GAAA	11, 14	15	11, 14
DYS449	AC051663	TTTC	30	27	30

In the optimization, samples with known genotypes for each Y-STR locus were amplified in singleplex PCR reaction. PCR products were then sequenced using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, Foster City, CA). The genotypes were verified by checking the final sizes and comparing the sequences obtained with the repeat motifs (Table 1).

2.3. PCR amplification and electrophoresis

Due to the high quality of Y-STRs that must be amplified in the same reaction, all selected Y-STRs were then organized by expected amplicon length and assigned into four different dye-labelling fluorochromes in order to achieve a balanced genotyping assay. One of the optimized primers for each Y-STR was labelled at the 5′ end with FAM, HEX, TAMRA or ROX (Applied Biosystems, Foster City, CA). The concentrations of primers and reaction elements of the final assay were optimized based on the genotyping profiles.

After optimization, the final 26plex typing system was performed in a 25 μL reaction volume including 12.5 μL reaction mix, 5 μL primer mix and 0.2–2 ng of template DNA. The reaction mix included 50 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.08 mg/mL bovine serum albumin (BSA), 0.5% Triton-X 100, 0.5% NP40, 1 U Q5 High-Fidelity DNA polymerase (Applied Biosystems, Foster City, CA). The final optimum parameters for PCR amplification were as follows: an initial denaturation step of 2 min at 96 °C, 30 cycles at 98 °C for 2 s, 60 °C for 40 s and 68 °C for 20 s with the MAX mode of GeneAmp 9700 (Applied Biosystems, Foster City, CA). The whole PCR amplification process can finish in 1 h.

PCR products were subsequently analyzed by mixing 1 μL of each amplified product, with 9 μL of a 17:1 mixture of Hi–Di formamide (Applied Biosystem, Foster City, CA) and ORG-500 Size Standard (75,80,100,140,160,175,180,200,225,250,275,300,330,360,390,445-,500) (PEOPLESPOTINC, Beijing, China) for electrophoresis. ORG-500 Size Standard was used as the internal lane standard. The mixture was denatured by heating 95 °C for 3 min and cooling at 4 °C for 3 min. The following detection were performed with AB 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) using filter set G5 and POP4 polymer (Applied Biosystems, Foster City, CA). Samples were injected electrokinetically for 5 s at 3 kV. The STR alleles were then separated at 15 kV by a run temperature of 60 °C. Genotyping data were then collected with GeneMapper ID v3.2.1 software (Applied Biosystems, Foster City, CA).

Control DNA of 9948 (Promega, WI, USA), XY5 (Qiagen, Hilden, Germany) and 007 (Applied Biosystems, Foster City, CA) human cell line samples were used as positive samples in the electrophoresis (genotyping data listed in Table 1). The nomenclature used was that of the latest recommendations for the DNA commission of the International Society of Forensic Genetics [9].

2.4. Allelic ladder

Allelic ladder comprising the most prevalent alleles at the 26 Y-STRs in the studied population is indispensable to the multiplex system. The PCR products of different genotypes at each Y-STR locus were cloned in plasmid. The successful clones of each allele were diluted, mixed, analyzed, and balanced to produce a single allelic ladder for each Y-STR. Those single allelic ladders were then mixed and balanced for a "cocktail" in appropriate portions [10]. The whole ladder-optimization step was performed based on the results of genotyping profiles. All alleles included in the in-house ladder were sequenced to confirm the number of repeats. When the in-house ladder was confirmed and optimized, Panel and Bin files for the Genemapper ID v3.2.1 were programmed. For the validation of the 26plex Y-STRs typing system, sizing precision testing was performed using the developed ladder.