



# Structure and polymorphism of 16 novel Y-STRs in Chinese Han Population

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**ABSTRACT.** Y-chromosome short tandem repeats (Y-STRs) are useful tools for identifying paternity origin and male-female mixed samples because of their male-specificity, haploid inheritance and relatively simplicity. We focused on novel Y-STRs deposited in the human Genome database from DYS708 to DYS726. We typed 16 male-specific Y-STRs from males of a Chinese Han population residing in Shanxi Province (north China), including DYS708-719, DYS721-723, and DYS726, but failed in typing DYS720, DYS724 and DYS725. The 16 Y-STRs, with mean gene diversity (GD) of 0.79, included three trinucleotide Y-STRs (711, 718, 719), nine tetranucleotide STRs (708, 709, 710, 712, 713, 715, 722, 723, 726) and four pentanucleotide repeat STRs (714, 716, 717, 721). DYS712, consisting of eight alleles, was the most informative STR in our population, with a GD of 0.843. The STRs were classified as simple STRs and complex STRs, according to their structures based on sequencing. Genetic indexes, including allele frequencies, haplotype distribution and male-specificity were determined. The Y-STRs, especially those male-specific, tetra- and penta-nucleotide, with only one copy on Y-chromosome, and relative simple structures, such as DYS709, DYS714, DYS715, DYS716, DYS718, DYS719, and DYS726, were suggested for the future forensic

DNA analysis, while DYS724 and DYS725 were not recommended for their multi-copy distribution. The population data provided putative Y-STRs for future genetic and forensic applications.

**Key words:** Y-chromosome short tandem repeats (Y-STRs); Shanxi; Population data; Chinese Han

## INTRODUCTION

The human Y chromosome is male-specific. The short tandem repeats (STRs) located on the Y chromosome, especially in the non-recombining region of the chromosome are passed on as a haplotype in a paternal mode of inheritance, providing discriminatory power in determining the male lineage of individuals. Being male-specific genetic markers, they are useful tools to distinguish the male DNA in material from sexual assault cases. However, Y-chromosome STRs (Y-STRs) are less polymorphic compared to autosome STRs because absence of recombination, thus making it less powerful for individual identification. This genetic property requires the establishment of genetic databases based on haplotypes of a group of Y-STRs in different populations in order to increase discrimination power. Y-chromosome polymorphisms are readily influenced by genetic drift processes and offer useful markers for genetic differences between related populations (Perez-Lezaun et al., 1999; Ge et al., 2010). Although over 700 Y-STRs have been named by now, only over 20 Y-STRs have been fully investigated and are commercially used, such as the Ampfl Y-filer kit (Applied Biosystems, USA) and the PowerPlex Y system (Promega). Because of the limitations of Y-STRs' specificity in geographic or population polymorphisms, more Y-STRs with more polymorphism and less population specificity are needed for forensic investigation purposes. Here, we investigated the "novel" released Y-STRs in Genome databank (GDB, [www.gdb.org](http://www.gdb.org), not available now) from DYS708 to DYS726. The Y-STRs were selected by the following criteria: 1) it was an STR system with small allele size ranging from 100 to 400 bp, which allows for multiplexing and degraded materials; 2) relatively simple structures, which would be easily typed by different laboratories; 3) the repeat-unit size should be  $\geq 3$  and a repeat count  $\geq 10$ , because less stutter bands and higher probability of variation would be expected as this criterium was set; 4) Y-chromosome specific, i.e., only one band appeared in male and no band in female. In this study, we collected blood samples for genotyping in a Chinese population from the Shanxi Province, located in the northern central part of China. The sequence structures and frequencies of each allele of the Y-STR were also confirmed in a Han population from the Shanxi Province. The polymorphisms of 16 Y-STRs from DYS708 to DYS726 were determined and their potential application in forensic casework was tested.

## MATERIAL AND METHODS

### Population

Whole blood samples were obtained from 120 unrelated healthy Chinese Han males living in Shanxi, a northern central Province of China, whose ancestors had lived in this region for at least three generations. Ethnic origin was enrolled by self-declaration. Blood of 20 female volunteers was collected to test Y-STR specificity.

## DNA extraction and quantitation

Genomic DNA was extracted using the hydroxybenzene-chloroform protocol (Zhang et al., 2004). Quantitation was done with a UV spectrometer, and DNA was stored at -20°C until used.

## PCR amplification and typing

The sequences of PCR primers were from GDB or redesigned using an online primer designing tool (<http://frodo.wi.mit.edu/primer3>) and synthesized by Takara (TaKaRa Bio Inc., Japan; primer sequences are listed in Table 1). The amplification reactions were performed in a total of 25 µL containing 0.5-1 µg DNA, 2.5 µL 10X PCR buffer, 200 µM dNTPs, 0.3 µM of each primer, and 1.5 U AmpliTaq DNA polymerase (5 U/µL, TaKaRa Bio Inc.). PCR amplification was carried out using a 9700 Thermal Cycler (Applied Biosystems). PCR was performed with the program as described: 95°C for 5 min, then 32 cycles at 95°C for 50 s, optimal annealing temperature for 45 s, 72°C for 45 s, and a final extension of 10 min at 72°C. The optimal annealing temperature was 59°C for DYS711, DYS712, DYS713, DYS719, DYS721, DYS723, DYS726, and 55°C for the rest of Y-STRs.

**Table 1.** Primers and structures of 16 Y-STRs in the Chinese Population from Shanxi.

Y-STR loci	Primers	Repeat-units	Repeats	Alleles
DYS708	F: AGTGTATCCGCCATGGTAGC R: CTGCATTTTGGTACCCATA	<i>(GATA)<sub>m</sub>(GACA)<sub>n</sub></i> ; <i>m</i> = 9-11, <i>n</i> = 7-10	Tetra	6
DYS709	F: GTTGCCATGGTTTCTTGCTT R: CGAACCTGCAAATTTGTTTAC	<i>(TTCT)<sub>4</sub>CTCT(TTCT)<sub>2</sub>(CTTTTCT)<sub>2</sub>CTT(TTCT)<sub>13-17</sub></i>	Tetra	5
DYS710	F: GAGGTCAAGGCTGCAAGAATCTATGA R: CATACTCTCCCTCCCTCTCTTTTTC	<i>(GAAA)<sub>m</sub>(GAG)<sub>n</sub>GA(GAAA)<sub>q</sub></i> ; <i>m</i> = 16-17, <i>n</i> = 6-8, <i>q</i> = 11-14	Tetra	5
DYS711	F: CAGAGCCCAGCACCTAGGTTAAGT R: TGCTGTCATTGTATCTTCTACTCC	<i>(CTT)<sub>m</sub>(CTC)<sub>1</sub>(CTT)CTC(CTT)<sub>3</sub>(CTCCTT)<sub>4</sub></i> <i>CTCCTA(CTT)<sub>q</sub></i> ; <i>m</i> = 26-34, <i>n</i> = 19-26	Tri	6
DYS712	F: CAAGAACAGCCTGGGTAACAGTG R: TATATGGTACAGCCCATGAACACTT	<i>(GAT)<sub>m</sub>(AGAC)<sub>n</sub></i> ; <i>m</i> = 13-17, <i>n</i> = 5-11	Tetra	8
DYS713	F: GTGCAAGCCAAGGGCTTTATAAGT R: CCTGGGTGACAGACTCCATCTTAAA	<i>(TCTT)<sub>1</sub>TC(TCTT)<sub>2</sub>(TCTG)<sub>1</sub>(TCTT)TTT</i> <i>(TCTT)TC(TCTT)<sub>n</sub>TT(TCTT)<sub>5</sub>CCT(TCTT)TC</i> <i>(TCTT)T(TCTT)</i> ; <i>m</i> = 16-22, <i>n</i> = 12-16	Tetra	6
DYS714	F: TATTAGGCCATCTTGCCAGC R: TTTTCTACCTATGATGCCCTTTG	<i>(TTTT)<sub>m</sub>(TCTTC)<sub>2</sub>(TTTTC)<sub>2</sub>(TCTTC)<sub>2</sub></i> <i>(TTTC)<sub>2</sub></i> ; <i>m</i> = 10-14	Penta	5
DYS715	F: ATGGTTGGAAGAAAGCATTGATGA R: CTAGGTAATTAGCTACCTAGTTAG	<i>(TAGA)<sub>12-16</sub></i>	Tetra	5
DYS716	F: TAAATCAGAATTCCTTTCCAATCCA R: TCTGGGTTTCAGAGTGGGATAATT	<i>(CACTC)<sub>6-7</sub>(CATTC)<sub>8-12</sub></i>	Penta	5
DYS717	F: GGCCGAGAGAATGGAATTGAT R: CCCGAACTTCAGCACTATGAAATG	<i>(TGTAT)<sub>2</sub>TAT(TGTAT)</i> <i>(TGTAT)<sub>m</sub>(TGTAT)<sub>n</sub></i> ; <i>m</i> = 5-7, <i>n</i> = 10-11	Penta	5
DYS718	F: CACAATGCAAAAAGAAAGAAGA R: GGAAGCAGCACACCAGCTT	<i>(TTA)<sub>14-17</sub></i>	Tri	4
DYS719	F: GAATGGGGAGGGATAACAA R: GGAGAAAAATCAATGCAGA	<i>(ATA)<sub>12-16</sub></i>	Tri	5
DYS721	F: GGGTGATAGAGGGAGGCTTCT R: CGGGCATGAGCTATTGAGTC	<i>(AAGGG)<sub>m</sub>N<sub>10</sub>(AAGGG)<sub>2</sub>N<sub>7</sub>(AAGCA)<sub>6</sub></i> ; <i>m</i> = 9-13	Penta	5
DYS722	F: GCAAAATTGTGAAGTACCAGCAAA R: GTGAACCTCTGCCAACCC	<i>(GAAA)<sub>m</sub>AAGA(GAAA)<sub>2</sub>A(GAAA)<sub>2</sub>GAGA</i> <i>(GAAA)<sub>2</sub></i> ; <i>m</i> = 13-17	Tetra	5
DYS723	F: GACAGGTGGATGCATAAATGG R: CCTATCTGGCATCTGTCTGC	<i>(GATA)<sub>2</sub>TAT(GATA)<sub>m</sub>GAT(GATA)<sub>1</sub></i> <i>GAT(GATA)<sub>7</sub></i> ; <i>m</i> = 10-14	Tetra	5
DYS726	F: GGGTAAACCTCTGAAGACCATAAC R: GAATGACAGACCAAGACTCTCTC	<i>(CTTC)<sub>2</sub>N<sub>13</sub>(CTTC)<sub>m</sub>N<sub>21</sub>(CTTT)<sub>3</sub></i> <i>T(CTT)<sub>2</sub></i> ; <i>m</i> = 11-14	Tetra	4

Italic repeat motif sequences indicate the variable repeats in our population.

PCR products were detected by 6% polyacrylamide gel electrophoresis (PAGE). The allelic ladder was made by mixing different PCR products from the same Y-STR. STR structures were confirmed by sequencing of each allele of the ladder. Allele name was assigned according to the International Society of Forensic Genetics (ISFG) guidelines for forensic Y-STR analysis (Gill et al., 2001; Gusmao et al., 2006).

### Statistical analyses

Haplotypes and allele frequencies were calculated by direct counting. Allele diversity [gene diversity (GD)] was calculated according to the Equation:

$$h = n (1 - \sum x^2) / (n-1), \quad (\text{Equation 1})$$

where  $n$  is the number of individuals, and  $x$  the allele frequency in the population. Standard error (SE) was calculated as follows:  $SE = \{(2/n)[\sum x^3 - (\sum x^2)^2]\}^{1/2}$ . Haplotype diversity was calculated as  $D = 1 - \sum P_i^2$ , where  $P_i$  represents the haplotype frequency. Bias correction was not done because the possibility that when each haplotype is observed once in a database, the corrected bias  $D$  would equal to 1 (Nei and Tajima, 1981; Dai et al., 2004; Budowle et al., 2009).

## RESULTS

### Informative loci and allele definition

A total of 84 alleles were found in our population group, ranging from 4 to 8 alleles for each Y-STR. Allelic frequencies of 16 Y-STRs are summarized in Table 2. The allelic number varied from four (DYS718, DYS726) to eight (DYS712). The mean GD of the 16 Y-STR was  $GD = 0.79 \pm 0.03$  (mean  $\pm$  SD), covering a range of 0.713 in DYS718 to 0.843 in DYS712.

GD in 14 Y-STRs was over 75%. The most frequent allele was allele 16 in DYS718 with a frequency of 0.358, followed by allele 19 in DYS726 (with a frequency of 0.325) and allele 14 in DYS719 (with a frequency of 0.308). Each male had one unique haplotype (Table 3).

Analysis of allelic sequences indicated that DYS709, DYS714, DYS715, DYS718, DYS719, DYS721, DYS722, DYS723, and DYS726 are simple Y-STRs, while DYS708, DYS710, DYS711, DYS712, DYS713, DYS716, and DYS717 are complex STR systems. The sequence data are shown in Table 4. They are identical to those in GDB and Genbank databases except for the motif repeats.

### Structures of the 16 Y-STRs

Among the 16 Y-STRs, there were three trinucleotide, nine tetranucleotide and four pentanucleotide STRs. DYS711, DYS718 and DYS719 belonged to the trinucleotide STRs, with the repeat sequence being (TTC), (TTA) and (ATA), respectively. DYS708, DYS709, DYS710, DYS712, DYS713, DYS715, DYS722, DYS723, and DYS726 belonged to the tetranucleotides, and their repeat sequences are listed in Tables 1 and 4. DYS714, DYS716, DYS717, and DYS721 were classified as pentanucleotide STRs. According to the structure of STR, they were classified as simple STRs, and complex STRs in accordance with Kayser's suggestions (Kayser et al., 2004). The numbers of repeat motif needed in the nomenclature system are shown Table 4.

**Table 2.** Allelic frequencies and statistical parameters regarding the 16 Y-STR loci of the Chinese Han population in Shanxi Province (N = 120).

Allele	DYS708	DYS709	DYS710	DYS711	DYS712	DYS713	DYS714		
18							0.192		
19					0.033		0.208		
20					0.158		0.217		
21		0.183			0.092		0.225		
22		0.283			0.217		0.158		
23		0.217			0.233				
24		0.175			0.133				
25	0.125	0.142			0.092				
26	0.142				0.042				
27	0.267								
28	0.208								
29	0.150								
30	0.108								
34			0.167						
35			0.217						
36			0.308						
37			0.183						
38			0.125						
40						0.100			
42						0.150			
44						0.192			
46						0.208			
48						0.225			
50						0.125			
54				0.167					
57				0.167					
59				0.175					
62				0.242					
64				0.133					
68				0.117					
GD	0.823	0.795	0.786	0.831	0.843	0.826	0.804		
SE	0.008	0.006	0.008	0.005	0.008	0.005	0.003		
Allele	DYS715	DYS716	DYS717	DYS718	DYS719	DYS721	DYS722	DYS723	DYS726
10									0.250
11									0.325
12	0.142					0.133			0.250
13	0.292					0.158			0.250
14	0.233			0.167		0.233			0.175
15	0.200	0.255		0.292		0.308			
16	0.133	0.183		0.358		0.167			
17		0.158		0.183			0.150		
18		0.192	0.267				0.225		
19		0.242	0.250				0.267	0.167	
20			0.150				0.217	0.233	0.192
21			0.192				0.142	0.275	0.192
22			0.141					0.200	0.217
23								0.125	0.233
24									0.167
25									
GD	0.785	0.802	0.793	0.731	0.787	0.795	0.793	0.803	0.745
SE	0.008	0.004	0.006	0.1	0.009	0.006	0.006	0.003	0.007

GD = gene diversity; SE = standard error.

## Simple STR

Simple microsatellites are those loci that consist of one type of repeated unit without interruptions of variant area. *DYS715*, *DYS718* and *DYS719* showed the simplest structures in this study, which present (TAGA), (TTA) and (ATA) as the repeat-unit, respectively. The loci *DYS709*, *DYS714*, *DYS721*, *DYS722*, *DYS723*, and *DYS726* have non-variant repeat block adjacent to the variant motif. The sequenced alleles of the six microsatellites showed that the variations were always due to the largest repeat block. Alleles of these simple STRs were named based on the total number of the repeat-unit located in variant and non-variant blocks (Table 4).

**Table 3.** Haplotypes of 16 Y-STR in the Shanxi Chinese population (N = 120).

No.	DYS708	DYS709	DYS710	DYS711	DYS712	DYS713	DYS714	DYS715	DYS716	DYS717	DYS718	DYS719	DYS721	DYS722	DYS723	DYS726
1	25	21	37	54	23	50	21	15	18	20	15	15	21	20	25	11
2	25	21	37	59	26	40	18	14	16	21	16	16	19	23	22	11
3	25	22	34	59	22	48	22	15	18	21	14	14	19	23	23	12
4	25	22	35	62	19	46	19	13	16	19	16	-14	19	22	24	12
5	25	22	37	57	25	44	20	13	15	22	17	14	17	21	22	12
6	25	22	38	57	23	48	18	15	17	18	17	14	20	22	23	11
7	25	22	38	59	25	42	20	12	19	21	16	13	18	23	25	13
8	25	22	38	64	20	40	20	15	18	19	14	13	17	21	23	11
9	25	22	38	68	22	50	18	13	15	20	15	15	20	21	21	11
10	25	23	36	54	25	46	19	14	18	19	15	11	19	21	23	11
11	25	23	36	64	24	50	21	13	19	19	17	14	19	20	23	12
12	25	23	36	64	26	44	19	13	15	20	15	14	17	19	20	11
13	25	24	35	62	23	44	21	14	15	20	17	15	20	22	24	14
14	26	21	34	59	23	44	19	12	17	18	16	16	20	19	21	13
15	26	21	34	59	21	42	19	12	15	18	16	15	18	22	21	13
16	26	21	36	57	23	44	22	16	16	21	16	13	19	23	24	12
17	26	22	34	54	24	48	22	15	19	18	17	13	21	19	25	11
18	26	22	35	62	23	42	20	14	19	20	14	15	18	20	24	13
19	26	22	36	59	23	50	18	12	19	22	16	16	21	19	21	14
20	26	22	37	54	26	48	21	16	19	20	16	15	20	21	20	14
21	26	22	38	62	22	44	21	14	19	19	15	12	18	23	21	14
22	26	23	35	64	22	42	21	14	19	19	15	15	18	20	25	13
23	26	23	36	59	20	46	19	15	16	21	15	16	19	23	23	11
24	26	23	37	62	21	50	22	13	18	19	15	16	19	20	21	13
25	26	24	36	62	25	46	18	15	16	21	15	15	19	23	25	11
26	26	24	37	54	22	48	22	15	19	18	14	15	18	21	25	12
27	26	24	37	62	22	46	20	12	18	21	16	12	20	22	24	11
28	26	24	37	64	23	44	20	12	18	19	15	14	17	21	22	14
29	26	25	34	62	23	50	22	16	16	22	16	14	19	22	25	12
30	26	25	35	57	22	44	22	16	16	18	16	16	20	22	23	12
31	26	25	37	62	19	42	20	13	18	19	14	13	18	22	23	11
32	26	25	38	59	20	46	21	15	18	21	16	15	19	21	24	14
33	27	21	34	68	24	44	21	13	19	19	15	14	18	22	22	12
34	27	21	35	59	21	50	18	16	17	18	15	12	19	21	20	13
35	27	21	36	64	24	48	20	13	16	21	16	14	20	20	24	13
36	27	21	37	57	23	46	21	12	17	18	16	13	21	22	23	12
37	27	21	37	62	25	46	21	13	18	19	16	15	19	19	20	14
38	27	21	38	62	23	46	19	15	18	19	15	13	18	19	22	13
39	27	22	34	62	20	48	22	15	18	19	16	16	19	22	24	11
40	27	22	35	57	21	48	21	13	19	21	16	15	21	19	21	13
41	27	22	36	54	21	46	18	15	15	18	17	14	21	19	22	12
42	27	22	36	59	23	42	19	16	18	22	16	14	20	22	22	14

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Table 3. Continued.

No.	DYS708	DYS709	DYS710	DYS711	DYS712	DYS713	DYS714	DYS715	DYS716	DYS717	DYS718	DYS719	DYS721	DYS722	DYS723	DYS726
43	27	22	37	68	23	40	18	15	19	21	15	13	21	21	23	11
44	27	22	38	57	21	48	22	14	19	21	16	13	19	20	22	11
45	27	23	34	64	24	40	21	16	19	22	16	15	20	21	22	14
46	27	23	34	68	24	48	19	16	16	22	16	16	18	22	25	14
47	27	23	35	62	26	48	19	16	18	19	15	15	19	23	22	14
48	27	23	37	68	25	48	19	12	18	22	14	14	20	23	20	12
49	27	23	38	68	22	48	20	15	16	21	16	15	18	22	22	13
50	27	24	34	54	20	46	20	13	16	19	15	14	19	19	20	13
51	27	24	35	62	22	48	18	15	19	19	14	13	20	21	23	12
52	27	24	35	64	20	44	20	13	18	19	15	14	19	21	21	12
53	27	25	35	57	22	50	18	12	19	20	17	15	20	21	21	13
54	27	25	36	68	23	48	20	12	18	22	16	15	21	22	25	12
55	27	25	36	68	26	44	19	12	19	19	15	15	18	22	21	13
56	27	25	37	57	22	48	19	13	17	18	17	15	21	19	21	12
57	28	21	35	57	21	46	18	15	15	20	17	15	17	19	23	12
58	28	21	35	62	20	48	20	15	19	18	14	12	19	20	23	11
59	28	21	36	62	25	46	18	12	15	20	17	16	20	22	25	12
60	28	21	36	62	23	46	20	13	18	22	15	12	19	20	24	11
61	28	21	38	64	20	42	21	15	16	19	15	12	19	22	22	11
62	28	22	34	62	24	50	18	12	15	21	17	15	19	19	25	12
63	28	22	34	62	22	44	21	14	17	21	17	14	17	20	24	12
64	28	22	35	54	24	42	18	14	17	20	15	16	17	22	21	14
65	28	22	35	59	23	44	21	15	15	18	17	15	20	21	22	14
66	28	22	36	59	22	48	19	14	18	21	16	15	20	23	22	12
67	28	22	36	64	25	46	22	13	19	19	15	15	17	20	25	13
68	28	22	37	62	22	46	22	13	17	19	15	15	18	20	22	13
69	28	22	38	68	23	50	21	13	15	18	16	16	17	20	22	11
70	28	23	34	59	24	50	21	14	15	20	16	14	18	21	23	12
71	28	23	35	68	22	44	21	13	15	20	17	12	21	20	22	12
72	28	23	36	54	23	44	19	13	15	22	17	14	19	21	23	13
73	28	23	36	57	24	44	22	13	16	20	16	13	21	23	23	13
74	28	23	36	62	20	42	21	14	15	18	16	15	17	20	22	12
75	28	23	36	62	22	44	19	14	15	18	16	12	18	19	23	12
76	28	23	37	57	22	50	18	16	15	20	16	12	17	19	22	11
77	28	23	38	59	23	44	18	13	17	20	17	16	17	21	24	13
78	28	23	38	62	21	46	19	16	15	18	14	14	20	19	23	11
79	28	24	36	54	20	42	18	13	15	20	16	13	17	20	24	13
80	28	24	36	54	23	42	22	14	19	18	14	12	17	21	23	11
81	28	24	36	54	20	48	19	16	15	18	17	16	20	20	21	12
82	28	24	36	62	22	42	19	13	15	19	14	16	18	23	23	11
83	28	24	37	59	22	46	22	12	15	18	16	14	18	19	20	12
84	28	24	37	64	19	42	22	16	18	19	15	15	19	20	21	14

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Table 3. Continued.

No.	DYS708	DYS709	DYS710	DYS711	DYS712	DYS713	DYS714	DYS715	DYS716	DYS717	DYS718	DYS719	DYS721	DYS722	DYS723	DYS726
85	28	24	38	62	24	44	20	13	16	18	16	12	19	20	22	12
86	28	24	38	68	22	44	20	15	17	19	15	16	19	21	23	12
87	28	25	36	54	22	50	20	14	15	20	16	15	17	21	22	14
88	29	21	34	64	20	44	19	13	19	19	15	13	18	19	23	11
89	29	21	36	57	23	48	20	15	19	18	14	14	18	20	21	11
90	29	21	36	57	24	42	20	14	17	20	14	12	18	21	21	13
91	29	21	37	54	22	40	21	13	18	21	15	16	19	20	20	11
92	29	21	38	57	25	48	18	14	17	22	17	12	18	20	23	14
93	29	22	34	54	22	46	21	12	17	19	15	12	21	22	22	13
94	29	22	35	64	20	50	20	14	15	19	14	16	18	19	21	11
95	29	22	36	64	25	40	22	14	16	22	14	14	21	21	23	12
96	29	22	37	54	21	48	20	14	19	18	16	12	18	19	21	11
97	29	22	37	57	25	46	22	14	16	22	16	15	19	23	22	14
98	29	22	38	54	22	48	19	14	15	18	17	13	17	20	24	13
99	29	23	35	59	22	42	19	13	16	21	15	15	20	21	23	12
100	29	24	35	57	23	44	20	12	17	21	17	14	17	20	24	12
101	29	24	36	68	23	40	21	14	18	18	16	15	20	21	23	14
102	29	24	37	57	21	42	20	15	19	21	14	15	20	21	23	14
103	29	25	36	59	23	42	21	13	17	19	15	15	18	22	24	13
104	29	25	36	59	20	46	19	14	16	21	14	15	19	20	25	13
105	29	25	36	62	20	48	22	16	16	19	15	14	17	21	20	11
106	30	21	34	57	21	44	18	14	19	19	16	14	18	20	21	13
107	30	22	35	59	23	48	22	14	17	18	16	14	21	22	24	12
108	30	22	35	68	23	46	20	15	18	18	16	15	20	19	24	13
109	30	22	36	54	20	46	18	13	16	20	15	12	19	23	20	11
110	30	23	34	62	24	40	19	14	18	19	15	13	18	20	23	11
111	30	23	35	54	24	48	19	16	19	22	16	16	21	21	25	14
112	30	23	35	59	20	40	21	12	16	21	16	13	20	21	20	12
113	30	23	35	64	23	46	18	16	18	18	16	16	20	21	20	14
114	30	23	36	64	22	50	21	13	17	18	15	16	21	21	20	13
115	30	24	35	57	20	42	20	15	19	22	14	16	20	21	23	12
116	30	24	36	54	20	46	18	13	15	20	16	13	20	23	22	13
117	30	25	34	59	23	48	21	13	19	22	14	13	18	21	23	14
118	30	25	34	68	24	42	20	14	16	18	14	13	20	22	20	12
119	30	25	34	68	19	44	18	13	19	19	15	14	19	20	24	13
120	30	25	35	62	24	44	21	13	15	18	17	16	21	22	22	12
GD	0.992															

GD = gene diversity.







Table 4. Continued.

Loci (ID in GenBank)	Allele (bp)	Sequences	Nomenclature
DYS722 [AC011289.4 (72629-72898)]	18 (291)	P1(21bp)(23bp)(aagg) <sub>10</sub> (10bp)(aagg) <sub>5</sub> (7bp)(aagca) <sub>6</sub> (120bp)P2(20bp)	m+2+2+2
	19 (294)	P1(21bp)(23bp)(aagg) <sub>11</sub> (10bp)(aagg) <sub>7</sub> (7bp)(aagca) <sub>6</sub> (120bp)P2(20bp)	
	20 (299)	P1(21bp)(23bp)(aagg) <sub>12</sub> (10bp)(aagg) <sub>7</sub> (7bp)(aagca) <sub>6</sub> (120bp)P2(20bp)	
	21 (304)	P1(21bp)(23bp)(aagg) <sub>13</sub> (10bp)(aagg) <sub>7</sub> (7bp)(aagca) <sub>6</sub> (120bp)P2(20bp)	
	Consensus structure	P1(25bp)aaigttaaacctcccaaaaaaaagaagaagaaagaaagaaagaaac(gaaa) <sub>m</sub> aagat(gaaa) <sub>1</sub> gagat(gaaa) <sub>2</sub> gctgaacacagatgaaggagaaacaacttgaatgatttcigagtttgagttgcaatctgtaggtctcagacacattgctP2(18bp)	
DYS723 [AC011289.4 (76324-76517)]	19 (258)	P1(25bp)(47bp)(gaaa) <sub>15</sub> aagat(gaaa) <sub>2</sub> at(gaaa) <sub>2</sub> gagat(gaaa) <sub>1</sub> (83bp)P2(18bp)	2+ m+ 1+7
	20 (262)	P1(25bp)(47bp)(gaaa) <sub>14</sub> aagat(gaaa) <sub>2</sub> at(gaaa) <sub>2</sub> gagat(gaaa) <sub>1</sub> (83bp)P2(18bp)	
	21 (266)	P1(25bp)(47bp)(gaaa) <sub>15</sub> aagat(gaaa) <sub>2</sub> at(gaaa) <sub>2</sub> gagat(gaaa) <sub>1</sub> (83bp)P2(18bp)	
	22 (270)	P1(25bp)(47bp)(gaaa) <sub>16</sub> aagat(gaaa) <sub>2</sub> at(gaaa) <sub>2</sub> gagat(gaaa) <sub>1</sub> (83bp)P2(18bp)	
	23 (274)	P1(25bp)(47bp)(gaaa) <sub>17</sub> aagat(gaaa) <sub>2</sub> at(gaaa) <sub>2</sub> gagat(gaaa) <sub>1</sub> (83bp)P2(18bp)	
	Consensus structure	P1(21bp)taaacatagat(gata) <sub>2</sub> tat(gata) <sub>m</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> aaacagatgaaaggatgaatgaatP2(20bp)	
	20 (194)	P1(21bp)(13bp)(gata) <sub>10</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> (51bp)P2(20bp)	
S72.6 [AC134879.3 (146076-146273)]	21 (198)	P1(21bp)(13bp)(gata) <sub>11</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> (51bp)P2(20bp)	m
	22 (202)	P1(21bp)(13bp)(gata) <sub>12</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> (51bp)P2(20bp)	
	23 (206)	P1(21bp)(13bp)(gata) <sub>13</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> (51bp)P2(20bp)	
	24 (210)	P1(21bp)(13bp)(gata) <sub>14</sub> gat(gata) <sub>1</sub> gat(gata) <sub>1</sub> (51bp)P2(20bp)	
	Consensus structure	P1(22bp)aaattgaaggaaacatacacacacagaaacacgtaaaat(ctt) <sub>3</sub> ctttctctctc(ctt) <sub>e</sub> tft(ctt) <sub>e</sub> ctctctctctct(ctt) <sub>3</sub> t(ctt) <sub>2</sub> gttcaattttP2(23bp)	
11 (205)	P1(22bp)(39bp)(cttc) <sub>1</sub> (13bp)(cttc) <sub>1</sub> tft(ctt) <sub>1</sub> (14bp)(ctt) <sub>1</sub> t(ctt) <sub>1</sub> (10bp)P2(23bp)		
12 (209)	P1(22bp)(39bp)(cttc) <sub>1</sub> (13bp)(cttc) <sub>1</sub> tft(ctt) <sub>1</sub> (14bp)(ctt) <sub>1</sub> t(ctt) <sub>1</sub> (10bp)P2(23bp)		
13 (213)	P1(22bp)(39bp)(cttc) <sub>1</sub> (13bp)(cttc) <sub>1</sub> tft(ctt) <sub>1</sub> (14bp)(ctt) <sub>1</sub> t(ctt) <sub>1</sub> (10bp)P2(23bp)		
14 (217)	P1(22bp)(39bp)(cttc) <sub>1</sub> (13bp)(cttc) <sub>1</sub> tft(ctt) <sub>1</sub> (14bp)(ctt) <sub>1</sub> t(ctt) <sub>1</sub> (10bp)P2(23bp)		

## Complex STR

Complex STR are those loci that contain more than one type of repeat-unit with/or without interruptions. DYS711, DYS713 and DYS717 comprised two variant motifs, which share the same repeat-unit, with/without the adjacent non-variant blocks. The aggregate number of the repeated unit in the interrupted variant motif and non-variant blocks was used for the allele name. With the repeat-unit of (CTT) for DYS711, (TCTT) for DYS713 and (TGAG) for DYS717, the variant and non-variant portions that comprised the allele diversity are listed in Tables 1 and 4. DYS708, DYS710, DYS712, DYS716 are more complicated STRs because they consist of different repeat-unit with (DYS710, DYS716) or without (DYS708, DYS712) interruptions. These four STRs comprise different repeat-unit with identical size, i.e., DYS708 consists of tetranucleotide variable repeat-unit of (GATA) and (GACA); DYS712 consists of repeated (AGAT) and (AGAC); DYS710 is comprised by three variant motifs (GAAA)<sub>m</sub>(GAGA)<sub>n</sub>GA(GAAA)<sub>q</sub>, m/n/q stands for 16-17, 6-8 and 11-14, respectively; DYS716 includes two pentanucleotide motifs of (CACTC) and (CATTC). Since the number of non-variant repeated units are less than three and separated by 20 nucleotides from the core variable motif in DYS726, they are excluded in the allele nomenclature. Alleles of DYS710 and DYS712 are named simply by the aggregation number of variable different repeat-unit. Non-variant repeats of DYS708 are considered for the allelic name because they are adjacent to the core variable motif blocks. We summarized the sequences comprising allele diversity and nomenclature of alleles by number of variable and non-variable repeat-unit in Table 4.

## Haplotypes and male specificity of 16 distinct STRs

A total of 120 distinct haplotypes consisting of the 16 Y-STRs are listed in Table 3. Since the haplotype of each male is unique, the unrelated males can be identified. The specificity for human males was determined by comparing DNA typing from 20 females and animals including pig, dog, chicken, rabbit, and rat. No PCR products were found using our primers and PCR parameters.

## Y-STRs failed to analysis

One typical Y-STR should show only one band when analyzed by PAGE due to its Y-chromosome-specific character. In our data, DYS724 and DYS725 showed more than two bands in their PCR products. When the products of DYS724 were visualized on PAGE, 32 individuals showed one band, 68 showed two bands, and 20 showed three bands. Besides, the polymorphism of DYS724 was limited, so we did not investigate it further. DYS725 also showed two bands and non-polymorphism in our population (data not shown). The information of DYS720 was lacking because we did not find suitable primers and amplification conditions for this STR. Due to the above-mentioned reasons, we did not conduct genotyping of these 3 Y-STRs in our collected samples.

## DISCUSSION

In the present study, we report the detailed information on allele frequencies and struc-

tures of novel Y-STRs deposited in Genome databank, which were named DYS708 to DYS726. They were successfully amplified and analyzed by designed primers and sequencing, except for DYS720, DYS724 and DYS725. The multiple bands may stem from duplication or multiple copies on the chromosomes. The Y-STRs with variable multiple bands mean that they are likely to be more informative than those STRs containing only one band, just like DYS389 and DYS385 (Komuro et al., 1998; Rolf et al., 1998; Niederstatter et al., 2005). However, multi-copies Y-STR would complicate the analysis of materials from sexual assault cases in forensic applications, such as the determination of the number of male suspects involved in the case. As a result, we do not suggest the two STRs, DYS724 and DYS725, to be involved into future Y-STR genotyping system because of their irregular allele numbers. In our population data, DYS712 had the highest GD, i.e., 0.843. Seven Y-STRs possessed a GD over 0.80, including DYS708, DYS711, DYS712, DYS713, DYS714, DYS716, and DYS723. Generally speaking, the alleles are relatively evenly distributed in this population since no allele frequency over 0.5 was found. These 16 Y-STR provide a highly haplotype diversity and would be more powerful if they are applied in groups or incorporated into commercial multiplex kits. Although the allele diversity and haplotypes of these STRs are not reported in other populations, the highly polymorphic could be expected in populations with similar genetic background, such as in Asia populations. Because Y-chromosome genetic markers are highly specific in a population group and dwelling region, the polymorphic data need to be studied in other ethnic populations. In fact, allele frequency information is one key discrepancy in different ethnic populations (Jarve et al., 2009).

The STRs were classified as simple STRs and complex STRs based on their structures in order to facilitate our analysis. Such classification may be changed in the future if more complex structure are found in other populations, just like the D21S11 (Urquhart et al., 1994). Even so, simple STRs are always preferred in future lineage analysis for their unambiguous typing. We named each STR allele according to ISFG's rules, as listed in Table 4. How to interpret repeated motifs is crucial to name an allele. One principle that should be followed is that allele name should have a mathematical relationship to the allele length. The most majority of Y-STR in this study were comprised of repeat-unit with the same size, except for DYS710 and DYS711. In this study, DYS710 was regarded as a tetranucleotide STR consisting of  $(GAAA)_m(GAGA)_nGA(GAAA)_q$  instead of  $(GAAA)_m(GA)_n(GAAA)_q$  because the sequences showed allele diversity with integral number of  $(GAGA)_m$  in this population. DYS711 was considered as a trinucleotide STR although it was composed by (CTT), (CTC) and (CTCCTT), because only  $(CTT)_m$  and  $(CTT)_n$  are related to the allele length.

To sequence each sample for each STR is an unacceptable huge work. However, compared with fully sequencing, high through-put automatic fragment analysis in forensic casework would compromise the STR polymorphism, because alleles with equivalent length but consisting of different sequences cannot be distinguished, particularly in those complex STR that have different repeat-units. To better understand the properties and sequence diversity of these STR for future genetic applications, thorough investigations in other populations are needed.

It should be noted that the primers of DYS709, DYS719, DYS722, and DYS723 have a good BLAST value of Pan troglodytes in Genbank. Although we did specificity test in pigs, dogs, chicken, rabbit, and rats, the species specificity of these STR in other species should be addressed in future studies, especially for forensic applications.

In summary, we identified 16 one-copy Y-STRs and 2 multi-copies Y-STRs with the allele diversities and structures in human males in the Chinese population. These Y-STRs, es-

pecially tetra- and penta-nucleotide Y-STRs, such as DYS709, DYS714, DYS715, DYS716, DYS718, DYS719, and DYS726 are suggested to be integrated in the multiplex systems after fully investigations in other populations and serve as the new members for forensic and genetic applications.

### Limitations of this study

As a preliminary screening study for suitable Y-STRs for forensic purposes, it did not involve a substantial population number and different ethnicities.

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