

RESEARCH ARTICLE

Diversity of five novel Y-STR loci and their application in studies of north Chinese populations

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Abstract

Y-chromosomal short tandem repeats (Y-STRs) show sufficient variability among individuals in a population and high degree of geographical differentiation, so their polymorphic character makes them especially suited for population genetic studies. In this study, five novel Y-STR loci were analysed in 174 samples from five minority populations residing in north China (Daur, Kazak, Xibe, Uighur and Kirgiz) to determine the diversity of these loci in north China and to evaluate their usefulness in population study. Ninety-seven haplotypes were constructed, with 30 in Daur, 24 in Kazak, 28 in Uighur, 27 in Xibe and 16 in Kirgiz. Sixty-six (68.04%) of them were unique. The R_{ST} showed that there was no significant difference in Daur and Xibe ($R_{ST} = 0.02231$, $P > 0.05$), while among the Kazak, Uighur and Kirgiz, who reside in northwest China, there were significant differences. These results showed that these five Y-STR loci were polymorphic in the five populations. The results of AMOVA showed that majority of the differences were found within populations. By R_{ST} , the relationships of the five populations were accordance with the historical records: Xibe migrated to Xinjiang during the Qing Dynasty, and Kazak, Uighur and Kirgiz have different ancestors.

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Introduction

The Y chromosome is inherited from father to son and the majority of its DNA lacks the potential for recombination. Therefore, all descendants of a male lineage share a common Y-chromosomal haplotype in the non-recombining region, unless a mutation alters the haplotype. Y-chromosomal STRs show sufficient variability among individuals in a population and high degree of geographical differentiation (Jobling and Tyler-Smith 1995). Thus, their polymorphic character makes them especially suited for forensic, genealogical and population genetic studies (de Knijff *et al.* 1997; Jobling *et al.* 1997; Schultes *et al.* 1999). The relationships and the microevolution of the male line of the different populations can be studied by using Y-STRs (Khodjet El Khil *et al.* 2001).

Y-STR haplotype reference database have been established for many populations around the world (Roewer *et al.* 2001; Kayser *et al.* 2002; Lessig *et al.* 2003; Willuweit and Roewer 2007). The minimal haplotype which consists of the loci DYS19, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393 and DYS385 can distinguish approximately 76–95% of male individuals in various populations (Roewer *et al.* 2001; Kayser *et al.* 2002; Schoske *et al.* 2004). Despite the utility of the minimal haplotype, additional Y-STR loci are required to improve the ability to distinguish different paternal lineages. Empirical studies of the other Y-STR loci are needed to provide an efficient marker set with sufficient power of discrimination. Recently, Lim *et al.* (2007) had analysed 52 new Y-STR loci in a worldwide panel and they found that DYS481, DYS488, DYS531, DYS576 and DYS589 are highly diversified (Lim *et al.* 2007).

In this study, we collected 174 unrelated male subjects who belong to five ethnic population of north China, Daur,

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Kazak, Xibe, Uighur and Kirgiz. These five ethnic populations are all from the Altaic linguistic family and belonged to different branches, in which Daur was Mongolic, Kazak, Uighur and Kirgiz were Turkic, and Xibe was Tungusic. We analysed five novel Y-STR loci (DYS481, DYS488, DYS531, DYS576 and DYS589) in these subjects to determine their diversity in north China.

Materials and methods

DNA samples

The Y-chromosomes of 174 unrelated male subjects, belong to five ethnic groups of north China, were analysed (table 1). Blood samples were collected from randomly selected individuals in each ethnic group. All subjects are resident in their own hometown, respectively, and the last three generations are endogamous for all subjects. Informed consent was obtained from all participants in this study. DNA was extracted from peripheral blood samples and anticoagulated with ACD, using the standard phenol-chloroform extraction method (Chomezynski and Sacchi 1987).

Genotyping of Y-STR and nomenclature

Five Y-STR loci (DYS531, DYS488, DYS589, DYS481 and DYS576) were genotyped in all individuals sampled. They were amplified in two multiplex PCR amplifications, multiplex 1 (DYS531 labelled with FAM, DYS488 labelled with JOE and DYS589 labelled with TAMR) and multiplex 2 (DYS481 labelled with JOE and DYS576 labelled with FAM). Only the forward primer was labelled with the fluorescent dye; the labelled primers were synthesized by Invitrogen (Shanghai, China). Primer sequences were as described in Kayser *et al.* (2004), and the primer details are listed in table 2. Multiplex PCR reactions contained 0.5 μ M of each primer, 200 μ M of each deoxynucleotide triphosphate (dNTP), 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM

MgCl₂, 1 U AmpliTaq Gold DNA polymerase and 5–10 ng of genomic DNA in a total volume of 20 μ L.

Amplification reactions were carried out in a Perkin Elmer GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, USA), and cycling conditions were as follows: initial denaturation at 94°C for 15 min, followed by 20 cycles of 90°C for 30 s, 70°C for 45 s and 72°C for 1 min (the anneal temperature decrease 1°C per cycle), 15 cycles of 94°C for 30 s, 50°C for 45 s and 72°C for 1 min and a final extension at 60°C for 45 min. Detection of the amplified products was accomplished with the ABI 3730 genetic analyzer (Applied Biosystems, Foster City, USA), and Y-STR alleles were named according to the number of repeat units they contain. The data files were generated by ABI PRISM GeneScan Analysis, and these files were analysed by the software Genotyper (Foster City, USA).

Data analysis

Haplotype and allele frequencies were calculated by direct counting. Gene or haplotype diversity (D) was calculated as $D = n(1 - \sum p_i^2)/(n - 1)$, where p_i is the frequency of the i th allele or haplotype, and n is the number of samples (Nei 1996). Genetic differentiation among population samples was estimated with the R_{ST} statistic using the software Arlequin 3.01 (<http://cmpg.unibe.ch/software/arlequin3/>). Genetic relationships among the five populations were further explored by analysis of molecular variance (AMOVA), as implemented in Arlequin 3.01 (<http://cmpg.unibe.ch/software/arlequin3/>). The significance level (α) of the analysis was 0.05. Multidimensional scaling (MDS) based on R_{ST} was carried out using PROXSCAL in SPSS 17.0 (SPSS, Chicago, USA).

Results

The five Y-STRs were typed in 174 males. Ninety-seven haplotypes were constructed. There were 30 haplotypes in Daur,

Table 1. The location of samples involved in present study.

Population	Sample size	Linguistic branches	Sampling place
Daur	38	Mongolic	Molidawa of inner Mongolia municipality
Kazak	39	Turkic	Yining city of Xinjiang municipality
Uighur	37	Turkic	Yining city of Xinjiang municipality
Xibe	34	Tungusic	Yili of Xinjiang municipality
Kirgiz	26	Turkic	Wuqia county of Xinjiang municipality

Table 2. The information of the Y-STR loci used in the study.

Marker	Repeats	Forward primer sequence	Reverse primer sequence
DYS481	CTT	JOE AGGAATGTGGCTAACGCTGT	G ACAGCTCACCAGAAGGTTCG
DYS488	ATA	JOE GGGGAGGGATAGCATTAGGA	G TACCCTGGTCCACTTCAACC
DYS531	AAAT	6FAM GACCCACTGGCATTCAAATC	G TGCTCCCTTTCTTTGTAGACG
DYS576	AAAG	6FAM TTGGGCTGAGGAGTTCAATC	G GGCAGTCTCATTTCCTGGAG
DYS589	TTATT	TAMR CATCCACATTGTTGCAAAGG	G TGACGAGTTAGTGGGTGCAG

Dye labels are indicated in bold character.

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24 in Kazak, 28 in Uighur, 27 in Xibe and 16 in Kirgiz. These haplotypes are listed in table 3. Sixty-six (68.04%) were unique. The most frequent haplotype surveyed here was 23-15-12-19-14 (DYS481-DYS488-DYS531-DYS576-DYS589), found in 11 individuals (6.3%). In these five populations, Daur had the highest haplotype diversity (0.9857) while Kirgiz had the lowest (0.9323); details are listed in table 4. Allele distribution and gene diversity data are detailed in table 5.

The pairwise R_{ST} results showed that there were no significant difference between Daur and Xibe ($P > 0.05$), while the differences between the other populations were significant (table 6). MDS based on the pairwise R_{ST} (figure 1) presented a direct view of the relationships of these populations. These five populations scattered in the plot, basically according to the geographical location, Daur and Xibe has a relatively closer relationship. There was no significant linguistic clustering.

Table 3. Y-STR haplotypes in 174 unrelated males of north China.

Haplotype	<i>n</i>	DYS481	DYS488	DYS531	DYS576	DYS589	Daur	Kazak	Uighur	Xibo	Kirgiz
H1	1	20	15	11	19	13	0	1	0	0	0
H2	1	21	16	13	17	15	0	0	1	0	0
H3	2	21	16	12	17	14	0	0	1	1	0
H4	1	21	15	12	16	14	0	0	0	1	0
H5	1	21	15	12	19	14	0	0	0	1	0
H6	1	21	15	12	19	13	0	0	0	1	0
H7	2	22	15	12	18	14	1	0	1	0	0
H8	3	22	16	12	19	14	1	0	0	0	2
H9	2	22	15	12	20	14	2	0	0	0	0
H10	1	22	15	12	19	15	0	1	0	0	0
H11	1	22	15	12	17	14	0	1	0	0	0
H12	1	22	15	13	19	15	0	0	1	0	0
H13	1	22	16	12	20	14	1	0	0	0	0
H14	1	22	15	11	18	14	0	0	0	1	0
H15	1	22	15	12	18	15	0	0	0	1	0
H16	1	22	15	12	20	15	0	0	0	1	0
H17	1	22	16	12	15	15	0	0	0	0	1
H18	1	22	15	11	15	13	0	0	0	0	1
H19	9	23	15	12	18	14	1	7	1	0	0
H20	3	23	15	12	20	15	3	0	0	0	0
H21	2	23	15	12	18	15	1	1	0	0	0
H22	1	23	15	12	19	15	0	1	0	0	0
H23	1	23	15	11	18	14	0	1	0	0	0
H24	1	23	14	12	19	14	0	1	0	0	0
H25	1	23	16	12	18	13	0	1	0	0	0
H26	11	23	15	12	19	14	0	6	0	0	5
H27	1	23	15	12	18	13	0	1	0	0	0
H28	1	23	17	12	18	14	0	1	0	0	0
H29	8	23	15	12	20	14	0	1	0	2	5
H30	1	23	15	12	20	12	0	0	1	0	0
H31	1	23	15	12	19	13	0	0	1	0	0
H32	1	23	15	12	14	13	0	0	1	0	0
H33	3	23	15	12	21	14	0	0	1	0	2
H34	1	23	15	13	18	14	0	0	1	0	0
H35	3	23	15	12	19	12	0	0	0	3	0
H36	3	23	16	12	19	14	0	0	0	3	0
H37	1	23	16	12	17	14	0	0	0	1	0
H38	1	23	15	13	20	14	0	0	0	0	1
H39	1	23	15	11	19	15	0	0	0	0	1
H40	1	23	16	12	18	14	0	0	0	0	1
H41	5	24	15	12	18	15	2	0	3	0	0
H42	2	24	15	11	19	15	1	0	0	1	0
H43	2	24	15	12	19	15	2	0	0	0	0
H44	1	24	12	12	18	14	0	1	0	0	0
H45	1	24	14	12	18	13	0	1	0	0	0

Table 3 (contd)

Haplotype	<i>n</i>	DYS481	DYS488	DYS531	DYS576	DYS589	Daur	Kazak	Uighur	Xibo	Kirgiz
H46	3	24	15	12	19	14	0	1	1	0	1
H47	6	24	15	12	18	14	2	3	1	0	0
H48	2	24	15	12	21	14	0	0	1	0	1
H49	5	24	15	12	20	14	0	0	4	0	1
H50	1	24	15	13	19	14	0	0	1	0	0
H51	2	24	15	12	20	13	0	0	2	0	0
H52	2	24	15	12	19	13	0	0	2	0	0
H53	1	24	15	12	18	13	0	0	1	0	0
H54	1	24	15	13	18	14	0	0	0	1	0
H55	1	24	16	12	18	14	0	0	0	1	0
H56	1	24	16	12	17	13	0	0	0	1	0
H57	1	24	16	12	19	14	0	0	0	1	0
H58	1	24	15	12	18	16	0	0	0	0	1
H59	1	25	15	12	16	15	1	0	0	0	0
H60	1	25	15	13	19	15	1	0	0	0	0
H61	1	25	16	13	21	14	1	0	0	0	0
H62	4	25	15	12	18	14	1	0	3	0	0
H63	1	25	16	11	18	14	1	0	0	0	0
H64	3	25	15	12	21	14	1	0	1	0	1
H65	1	25	15	11	17	15	0	1	0	0	0
H66	1	25	15	12	20	14	0	0	1	0	0
H67	1	25	15	12	17	14	0	0	1	0	0
H68	1	25	15	11	18	13	0	0	1	0	0
H69	1	25	15	13	19	14	0	0	1	0	0
H70	1	25	15	12	19	15	1	0	0	0	0
H71	2	25	15	12	18	13	0	0	0	2	0
H72	1	25	15	12	19	14	1	0	0	0	0
H73	1	25	15	11	19	13	0	0	0	0	1
H74	1	26	15	12	20	12	1	0	0	0	0
H75	6	26	15	11	18	13	1	2	1	1	1
H76	1	26	14	12	18	14	1	0	0	0	0
H77	1	26	15	11	18	15	0	1	0	0	0
H78	1	26	15	10	18	13	0	1	0	0	0
H79	3	26	15	12	18	14	0	2	0	1	0
H80	2	26	15	11	18	14	0	0	0	2	0
H81	1	26	15	12	19	14	0	0	0	1	0
H82	2	27	15	12	18	15	1	0	1	0	0
H83	1	27	15	12	19	14	1	0	0	0	0
H84	2	27	15	12	18	14	0	1	0	1	0
H85	2	27	15	11	18	12	2	0	0	0	0
H86	1	27	15	11	18	13	0	0	0	1	0
H87	1	28	15	12	18	15	1	0	0	0	0
H88	1	28	15	12	18	12	1	0	0	0	0
H89	2	28	15	11	19	15	2	0	0	0	0
H90	1	28	15	11	21	14	1	0	0	0	0
H91	1	28	15	10	17	14	1	0	0	0	0
H92	1	28	15	11	19	16	0	0	1	0	0
H93	1	28	15	11	19	14	0	0	0	1	0
H94	1	29	15	12	19	14	0	1	0	0	0
H95	1	29	15	11	20	14	0	0	0	1	0
H96	1	29	15	11	20	13	0	0	0	1	0
H97	1	30	15	11	19	10	0	0	0	1	0
Population size							38	39	37	34	26

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Table 4. Haplotype diversity of the five populations studied.

Population	Sample size	Number of haplotypes	Haplotype diversity
Daur	38	30	0.9857
Kazak	39	24	0.9824
Xibe	37	28	0.9665
Uighur	34	27	0.9850
Kirgiz	26	16	0.9323

Table 5. Y-STR allele distribution, frequency and gene diversity(GD) in north China.

Locus	Allele	Frequency					
		Daur	Kazak	Uighur	Xibe	Kirgiz	
DYS481	20	0.0	2.6	0.0	0.0	0.0	
	21	0.0	0.0	5.4	11.8	0.0	
	22	13.2	5.1	5.4	8.8	15.4	
	23	13.2	53.8	16.2	26.5	57.7	
	24	18.4	15.4	43.2	14.7	15.4	
	25	21.1	2.6	21.6	5.9	7.7	
	26	7.9	15.4	2.7	14.7	3.8	
	27	10.5	2.6	2.7	5.9	0.0	
	28	15.8	0.0	2.7	2.9	0.0	
	29	0.0	2.6	0.0	5.9	0.0	
	30	0.0	0.0	0.0	2.9	0.0	
GD		0.8507	0.776	0.6738	0.7522	0.6369	
DYS488	12	0.0	2.6	0.0	0.0	0.0	
	14	2.6	5.1	0.0	0.0	0.0	
	15	86.8	87.2	94.6	76.5	84.6	
	16	10.5	2.6	5.4	23.5	15.4	
	17	0.0	2.6	0.0	0.0	0.0	
GD		0.2415	0.2834	0.22	0.081	0.2708	
DYS531	10	2.6	2.6%	0.0%	0.0%	0.0%	
	11	21.1	15.4	8.1	29.4	15.4	
	12	71.1	82.1	78.4	67.6	80.8	
	13	5.3	0.0	13.5	2.9	3.8	
GD		0.4595	0.4642	0.3102	0.3709	0.3354	
DYS576	14	0.0	0.0	2.7	0.0	0.0	
	15	0.0	0.0	0.0	0.0	7.7	
	16	2.6	0.0	0.0	2.9	0.0	
	17	2.6	5.1	8.1	8.8	0.0	
	18	42.1	61.5	37.8	35.3	11.5	
	19	26.3	30.8	21.6	38.2	38.5	
	20	18.4	2.6	21.6	14.7	26.9	
	21	7.9	0.0	8.1	0.0	15.4	
	GD		0.8016	0.5439	0.5371	0.7703	0.7661
	DYS589	10	0.0	0.0	0.0	2.9	0.0
12		10.5	0.0	2.7	8.8	0.0	
13		2.6	17.9	24.3	20.6	11.5	
14		44.7	69.2	54.1	58.8	76.9	
15		42.1	12.8	16.2	8.8	7.7	
16		0.0	0.0	2.7	0.0	3.8	
GD		0.5249	0.5128	0.4845	0.6407	0.4031	

According to the geographical location we divided the five ethnic groups into two groups, northeast and northwest. Based on the historical records and the MDS results, Xibe was placed into the northeast group. Then we carried out

AMOVA on the two groups. The results showed that the majority of the variation was found within populations. The difference between northeast and northwest was not significant ($P > 0.05$). Then, we divided the five populations into three

Table 6. The pairwise R_{ST} among the five populations.

Population	Daur	Kazak	Xibe	Uighur	Kirgiz
Daur	–	0.07024**	0.02231	0.02469*	0.07910**
Kazak	0.07024**	–	0.06211**	0.03071**	0.05447**
Xibe	0.02231	0.06211**	–	0.02930	0.06394**
Uighur	0.02469*	0.03071**	0.02930	–	0.02785*
Kirgiz	0.07910**	0.05447**	0.06394**	0.02785*	–

* $P < 0.05$, ** $P < 0.01$, number of permutations = 110.

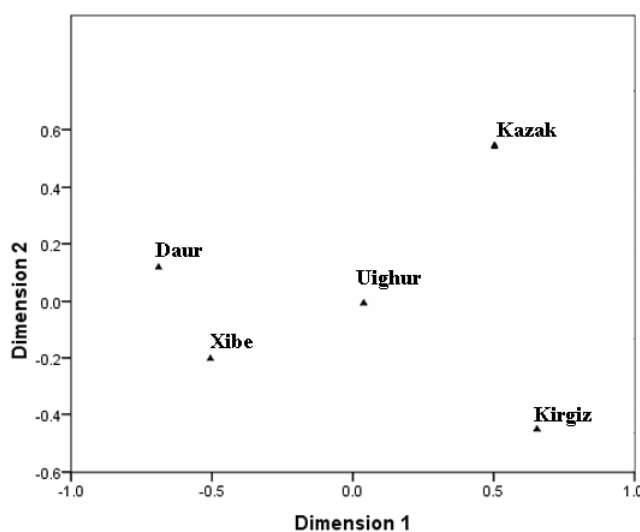


Figure 1. Relationships among five populations represented by MDS.

groups according to the linguistic family: Mongolic (Daur), Turkic (Kazak, Uighur and Kirgiz) and Tungusic (Xibe) to conduct the AMOVA. The results shows that majority of the differences were found within populations, and there were no significant difference among the linguistic branches (table 7).

Discussion

Human Y-linked polymorphisms in the non-pseudo-autosomal portion of the Y-chromosome are inherited as haplotypes within paternal lineages. This part of Y-chromosome lacks recombination, which is as same as mitochondrial DNA, and passed down from father to son unchanged except by the gradual accumulation of mutations. The use of Y-chromosome polymorphisms, especially Y-STRs, has become commonplace in a variety of forensic situations for mixture analysing and paternity testing (Jobling *et al.* 1997; Hall and Ballantyne 2003; Hanson and Ballantyne 2004; Diederich *et al.* 2005), as well as in human evolutionary studies and population history research (Jobling *et al.* 1997; Parkin *et al.* 2006; Kumagai *et al.* 2007; Mertens *et al.* 2007; Huang *et al.* 2008). The STR polymorphisms can be used to construct highly discriminative Y-haplotypes, which will serve to isolate the male ancestor of different populations.

In this study, we genotyped five novel Y-STR loci in five populations, the results showed that the gene diversity ranged from 0.081 at DYS488 (Uygur) to 0.8507 at DYS481 (Daur) and the haplotype diversity was above 90% in these five populations. The results revealed that the set of five Y-STR loci had a high level of diversity in north China, although they were not enough for the forensic discrimination.

The results of AMOVA showed that majority of the variation were found within populations, not only when groups were based on geographical location but also when the groups were based on linguistic family. These results also indicated that the set of loci has a high diversity in these populations, and the north populations had a relatively closer relationship among themselves.

By pairwise R_{ST} , we found the value of R_{ST} between Xibe (the population of northwest China) and Daur (the population of northeast China) was lower than that of Xibe with the other northwest populations. The difference between Xibe and Daur was not significant ($P > 0.05$), while there was significant difference among the other northwest Chinese populations. The MDS plot was also consistent with this clustering. These results are in accordance with the historical record. Daur is a population which lives in Heilongjiang, and Xibe is the descent of Xianbei who belonged to the eastern

Table 7. Summary of results of two separate AMOVAs in the five populations.

Basis of division	Groups	Percentage of variation		
		Within populations	Among populations within groups	Among populations
Geography	Northeast, northwest	92.19	3.30	4.51*
Linguistic family	Mongolic, Turkic, Tungusic	92.65	2.84	4.51*

* $P > 0.05$.

Hu system of ethnic groups in ancient northeast China. In 1764 AD, 1020 soldiers and some 4000 dependants moved to west Xinjian to defend the borders (Ruofu 1994). The significant differences of the R_{ST} value ($P < 0.05$) among the other northwest populations is in concordance with the studies of Xiao Chunjie (2000) and Xue (personal communications). The three northwest populations are quite separate, and they have different origins. The ancestors of Uygur lived in the area of lake Baikal and the region between lake Balkhash and the Ertix river. The original meaning of Kazak is 'refugee'. In 1456 AD, group of people from the Uzbek Khanate migrated to the east and became known as Kazak. The Kirgiz are the descendents of the Jian Kuen mixed with some parts of the Khitan, Turkic, Mongolian and Uygur ethnic groups (Ruofu 1994). Thus, there was significant difference among these three populations.

The five Y-STR loci have a fine diversity in these five populations, and they were suited for the study of population genetics and evolution. But they are not enough to get a precise result just by these five loci, so we will detect some other Y-STR loci in these populations, at the same time, we will add other ethnic populations to get the reliable results.

Our results showed that these five Y-STR loci have a good polymorphism in the five populations. By the R_{ST} analysis, there was no significant difference between Daur and Xibe, while there were significant differences among the other northwest Chinese populations. The results are accord with the other studies and the historical record.

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