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Announcement of Population Data

Haplotype analysis of 17 Y-STR loci in a Japanese population

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Abstract

We analyzed 17 Y-STR loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS456, DYS458 and DYS464a/b/c/d) in 252 Japanese males using three multiplex PCR typing systems. Two variants were found at DYS385a/b. A total of 244 different haplotypes were observed, of which 239 were found in single individuals. The haplotype diversity for the 17 loci was 0.996.

Keywords: Y chromosome; STRs; Haplotypes; DYS385; Mutation; Japanese; Population data

Population: DNA samples from 252 unrelated males, 10 pairs of father-son dyad, three pairs of male sibling and five females living in Iwate Prefecture, Northeast Japan were studied.

Extraction: DNA of each individual was extracted from blood using the conventional phenol/chloroform method or a PUREGENE® Genomic DNA Purification Kit (Gentra Systems, USA).

PCR: The 17 Y-STRs were amplified in three PCR multiplex reactions; 5-plex PCR (DYS389 (0.6 μM of each primer), DYS391 (0.2 μM), DYS392 (0.4 μM) and DYS393 (0.2 μM)), 4-plex PCR (DYS19 (0.8 μM), DYS385 (0.6 μM) and DYS390 (0.4 μM)) and 8-plex PCR (DYS438 (0.6 μM), DYS439 (0.6 μM), DYS456 (0.4 μM), DYS458 (0.4 μM) and DYS464 (0.6 μM)). Primers were synthesized using sequences reported in the literature [1–3], and the forward primers were labeled with FAM, HEX or TET at 5′ terminal. PCR reaction mixtures contained 1–2 ng genomic DNA and 5 μL PCR Master Mix (Promega Co., USA) in a total volume of 10 μL. Thermal cycling was performed according to the manufacturer's instruction for PE2400 of PowerPlex® Y system (Promega). The two loci of DYS385a and DYS385b were individually amplified using primers designed from the single copy sequence between the two 190 kb copies containing DYS385a and DYS385b [4], and the primer sequences and amplified fragment sizes are listed in Table 1. The PCR products of 0.9 kb in length were amplified in a 10 μL mixture containing 0.5 μM of each primer, 5 μL PCR Master Mix (Promega) and 2 ng genomic DNA. The cycle condition and PCR for genotyping of each DYS385 locus were performed as described elsewhere [1]. PCR reactions for fragments of 1.8, 4.1 and 8.4 kb in length were carried out in a 25 μL reaction volume, containing 1× LA PCR Buffer II with 2.5 mM $MgCl_2$, 320 μM of each dNTP, 2 U TaKaRa LA Taq (Takara Bio Inc., Japan), 0.6 μM of each primer, and 20 ng genomic DNA. The cycle conditions were as follows: 98 °C for 1 min, followed by 30 cycles of 98 °C for 10 s, 68 °C for 2–8 min, and a 7 min extension at 72 °C.

Genotyping: The PCR products were electrophoresed with a 5% denaturing polyacrylamide gel prepared with Long Ranger® gel solution on the ABI PRISM 377 DNA Sequencer. Fragment size was determined with GeneScan® v.3.1 and ILS600 (Promega) as the internal size standard. All alleles were designated according to the number of repeat units as recommended by the DNA commission of the International Society for Forensic Genetics [5].

Sequencing: Several samples were directly sequenced on ABI310 Genetic Analyzer or ABI377 DNA sequencer using the DYEnamic® ET Terminator Cycle Sequencing Kit (GE Healthcare Bio-Science Co., USA) to verify the allele sequences. Furthermore, PCR products in multi-locus STR were cloned into a T vector using *p*GEM-T™ Vector System I (Promega) and the inserts were sequenced with M13 forward primer.

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Table 1
Primer sequences for individual amplification of DYS385

Locus	Primer	Sequence	Amplified fragment size (kb)	Sequence position
DYS385a	DYS385-1A	5′-CAGGAGGCTTAGGAAGGCTAA-3′	0.9	AC022486 (nt127497–127517)
	DYS385-2L	5′-ATGCCCGCTACTCAATGTTC-3′		AC022486 (nt126590–126609)
DYS385b	DYS385-1B	5′-TGCTGTTAAGCACCATCCAG-3′	0.9	AC007379 (nt10819–10838)
	DYS385-2L	5′-ATGCCCGCTACTCAATGTTC-3′		AC007379 (nt11756–11775)
DYS385a	DYS385-1A	5′-CAGGAGGCTTAGGAAGGCTAA-3′	1.8	AC022486 (1nt27497–127517)
	Y1488	5′-CCTTTCAGTGAGGGGATTACAG-3′		AC022486 (nt125688–125709)
DYS385b	DYS385-1B	5′-TGCTGTTAAGCACCATCCAG-3′	1.8	AC007379 (nt10819–10838)
	Y1488	5′-CCTTTCAGTGAGGGGATTACAG-3′		AC007379 (nt12656–12677)
DYS385b	SHGC78789	5′-AATATGAAGTGCCCATGCAGAAT	4.1	AC007379 (nt8759–8781)
	Y1488	5′-CCTTTCAGTGAGGGGATTACAG-3′		AC007379 (nt12656–12677)
DYS385a	DYS385-1A	5′-CAGGAGGCTTAGGAAGGCTAA-3′	8.4	AC022486 (nt127497–127517)
	DYS207F	5′-TTTCACACTGTTAGAAATCAGGC-3′		AC022486 (nt117940–117962)
DYS385b	DYS385-1B	5′-TGCTGTTAAGCACCATCCAG-3′	8.4	AC007379 (nt10819–10838)
	DYS207F	5′-TTTCACACTGTTAGAAATCAGGC-3′		AC007379 (nt20403–20425)

Quantification of DNA concentration: The concentration of PCR products was quantified by the end-point method [6] using the PicoGreen® dsDNA Quantification Kit (Molecular Probes, USA). The 4.1 kb fragments containing 2.3 kb of single copy domain between DYS385a and DYS385b were amplified. In a preliminary experiment amplifying the 4.1 kb fragments by PCR using 1 and 2 μL of 5 ng/μL DNA with thermal cycling of 24–32 cycles, quantitative amplification was observed by thermal cycling at 26–30 cycles. Therefore, the PCR products were quantified by thermal cycling of each 26, 28 and 30 cycles using 1 μL of 5 ng/μL genomic DNA.

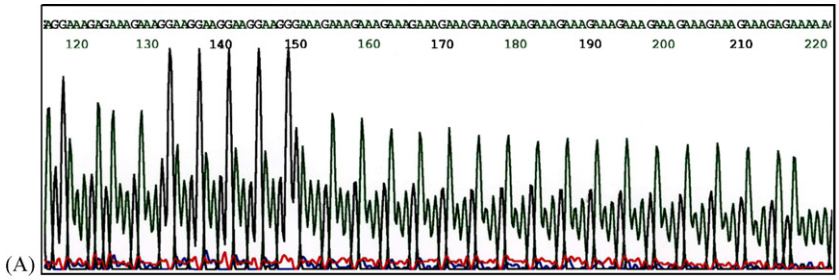
Quality control: We used the SRM 2395 Human Y-chromosome DNA Profiling Standard (National Institute of Standards & Technology, USA) for quality assurance.

Results: The data are shown in Table 2 and Fig. 1.

Analysis of data: The allele frequencies were calculated by the simple gene count method. Haplotype and gene diversity was calculated according to the formula shown by Nei [7]. AMOVA analysis and calculation of F_{ST} values were performed using Arlequin 3.0 [8].

Access to data: rkumagai@forensic.iwate-med.ac.jp.

Other remarks: Among the 252 unrelated male individuals a total of 244 different haplotypes were observed, of which 239



(A)

(B)

DYS385 forward primer

1	Allele 16	AGCATGGGTGACAGAGCTAGACACCATGCCAAACAACAACAAAGAAAAGAAATGAAATTC
2	Variant	AGCATGGGTGACAGAGCTAGACACCATGCCAAACAACAACAAAGAAAAGAAATGAAATTC
1	Allele 16	AGAAAGGAAGGAAGGAAGGAGAAAGAAAGTAAAAAAGAAAGAAAGAGAAAAAGAGAAAAA
2	Variant	AGAAAGGAAGGAAGGAAGGAGAAAGAAAGTAAAAAAGAAAGAAAGAGAAAAAGAGAAAAA
1	Allele 16	GAAAGAAAGAGAAGAAAGAGAAAGAGGAAAGAGAAAGAAAGGAAGGAAGGAAGGAAGGAA
2	Variant	GAAAGAAAGAGAAGAAAGAGAAAGAGGAAAGAGAAAGAAAGGAAGGAAGGAAGGAAGG ----
1	Allele 16	GGGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGA
2	Variant	---- GAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGA
1	Allele 16	AAGAGAAAAAGAAAGGAGGACTATGTAATTGGAATAGATAGATTATTTTTTAAAATATTT
2	Variant	AAGAGAAAAAGAAAGGAGGACTATGTAATTGGAATAGATAGATTATTTTTTAAAATATTT
1	Allele 16	TTATTACCTTTACAGTTTTTTTAAATGCCGCCATTTCAGAAAGAAATCTGGTCAGCAGCC
2	Variant	TTATTACCTTTACAGTTTTTTTAAATGCCGCCATTTCAGAAAGAAATCTGGTCAGCAGCC
1	Allele 16	CTTACCAGCTTTACCTAGCATCCC
2	Variant	CTTACCAGCTTTACCTAGCATCCC

DYS385 reverse primer

Fig. 1. (A) Electropherogram of the variant observed for DYS385a locus. (B) Alignment of top strands from allele 16 (1) and the variant (2) for the DYS385 locus. The upstream AAGG unit (underlined section) of the STR unit of GAAA indicated by bold face was five times of the variant, instead of six times in the specimen of allele 16. The deletion of 4 bp made the fragment size of the variant equal to that of allele 15.