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Brief Communication

# Multiplex PCR for 18 X-chromosomal STRs in Japanese population

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ABSTRACT

X-chromosomal STR (X-STR) polymorphisms are particularly useful in complex cases of kinship testing involving inheritance through female subjects. An X-chromosomal multiplex amplifying 18 STRs in one single PCR reaction was developed and optimized in this study. The multiplex system included the DXS7424, GATA172D05, HPRTB, DXS8377, GATA31E08, DXS6810, DXS7423, DXS981, DXS6795, DXS6803, DXS6789, DXS6800, DXS6809, DXS7133, DXS7132, DXS9902, DXS101 and DXS6807 loci, which are distributed over the whole X-chromosome. It was designed as a potential first option in determining recombination within the whole X chromosome in kinship testing. Allele frequencies were obtained from samples from 378 male and 175 female Japanese individuals, all unrelated. The sizes of the amplified products ranged from 82 to 297 bp. The combined power of discrimination of the 18 loci was 0.999999999999997 in females and 0.9999999992 in males. A case is presented in which this system allowed considerable efficacy in reaching a solution. The present multiplex system amplified the largest number of loci among the X-STR multiplex systems tested, indicating its potential in personal identification and determining kinship.



## 1. Introduction

X-chromosomal STR (X-STR) polymorphisms are an important tool in kinship testing in forensics [1–3]. They are particularly useful in complex cases in which autosomal STR analysis is insufficient to determine blood relationships with any degree of certainty and Y-chromosomal or mitochondrial DNA polymorphisms are of no use or inconclusive. If part of an X chromosome is inherited, increasing the number of polymorphic loci to be analyzed in the inherited region would increase the degree of certainty in determining kinship. In such cases, we believe it would be of benefit if the whole X chromosome could be searched for as many STR markers as possible using only a small number of PCR amplifications.

We have already reported a method of amplifying 15 X-STR loci to construct a database for many common loci in the Japanese population [4]. However, in the course of applying X-STR polymorphisms to actual cases, we encountered many instances in which we needed to look for inherited regions taking into consideration recombination on X chromosomes. Moreover, it was necessary to obtain results using only small amounts of DNA.

Therefore, in this study, an 18-locus simultaneous multiplex system was developed by adding 3 loci (DXS9902, DXS6795, and DXS6810) on the short arm of the X chromosome to the previous 15-locus multiplex system [4]. This was achieved by improving the primer sequences and optimizing PCR conditions. New multiplex-PCR system is distributed over the whole X-chromosome. It was designed as a potential first option in determining recombination within the whole X chromosome in kinship testing. A large number of individuals were examined to establish reference databases in the Japanese population and a case is presented in which the present multiplex system was effective.

## 2. Materials and methods

### 2.1. Samples

Genomic DNA was extracted from blood samples obtained from 546 unrelated Japanese individuals (371 men and 175 women). Informed consent was obtained from all donors, including those individuals in the case samples. This study was approved by the Ethics Committee of Tokyo Dental College (approval Nos. 202 and 204). Isolation of genomic DNA was performed as described previously [4].

### 2.2. PCR amplification and typing of X-STRs

Eighteen X-STR loci (DXS7424, GATA172D05, HPRTB, DXS8377, GATA31E08, DXS6810, DXS7423, DXS981, DXS6795, DXS6803, DXS6789, DXS6800, DXS6809, DXS7133, DXS7132, DXS9902, DXS101 and DXS6807) were examined for polymorphisms (Fig. 1). Among these, 15 loci were the same as those in our previous study

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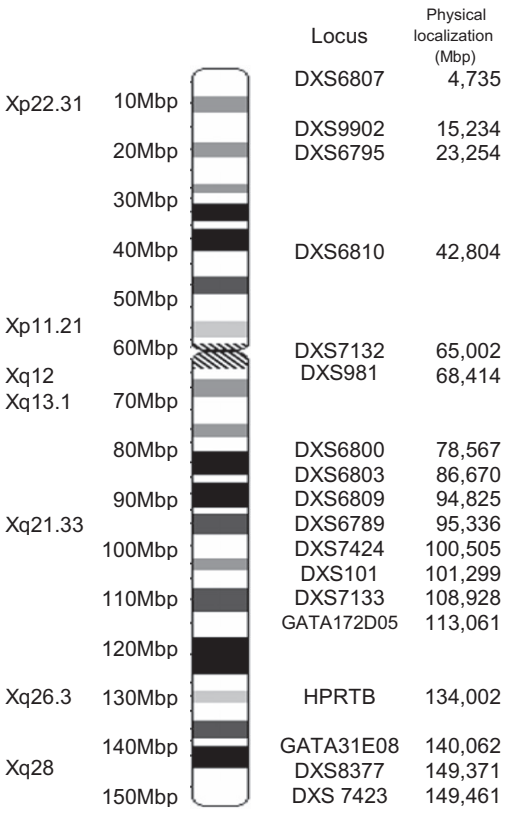


**Fig. 1.** Positions of 18 X-chromosomal loci on chromosome X ideogram.

[4]. Although these loci were distributed over the whole X chromosome, the multiplex did not cover the short arm of the X chromosome, making it impossible to screen for recombination there. Therefore, three additional loci (DXS9902, DXS6795, and DXS6810) were selected on the short arm of the X chromosome [X-STR org (http://www.chrx-str.org)].

The primer sequences, concentrations used in the multiplex, type of labeled dye and range of amplified fragment sizes are listed in Table 1. Some of the primer sequences for the 15 loci used in the previous study [4] were changed slightly and primer sequences for the additional three loci newly constructed. Multiplex PCR was performed in 25 μl reaction mix containing the following: 1 ~ 5 ng genomic DNA, 10 mM Tris–HCl at pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 200 μM dNTP, 1.5 U AmpliTaq Gold (Applied Biosystems) and an appropriate volume of each primer (Table 1). The PCR temperature profile was as follows: 11 min at 95 °C followed by 50 s at 95 °C and 105 s at 58 °C for 27 cycles, with a final extension at 60 °C for 60 min. Twelve microliters Hi–Di formamide (Applied Biosystems) and 0.5 μl GeneScan-500 LIZ internal size standard were added to each PCR product. Electrophoresis was performed using the ABI PRIZM 310 Genetic Analyzer (Applied Biosystems). Fragment sizes were automatically determined using the GeneScan Analysis software 3.1 (Applied Biosystems) and results analyzed using the Genotyper ver. 2.5 (Applied Biosystems). Genotyping was performed through comparison with sequenced samples and DNA control reference samples 9947A (Applied Biosystems) and K562 (Promega) to validate the present typing protocol using multiplex X-chromosomal STRs [9].

### 2.3. Sequencing analysis

For a comparison with the established allele nomenclature of DXS9902, DXS6795, and DXS6810, several allelic PCR products from hemizygous male participants were directly sequenced. Amplicons were purified with the PureLink PCR purification kit (Invitrogen) according to the manufacturer's instructions. The PCR for sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Excessive dye was removed using Performa DTD Gel Filtration Cartridges (EdgeBio). Sequence analysis was performed on an ABI PRISM 3100 automated sequencer (Applied Biosystems).

### 2.4. Statistical analysis

The chromosomal location of the 18 markers was determined by querying the NCBI map viewer. Observed heterozygosity was calculated using female data with the PowerStatsV12 software (http://www.promega.com). Polymorphism information content, power of discrimination in females, power of discrimination in males, and power of exclusion were also calculated with the PowerStatsV12 software. The Hardy–Weinberg equilibrium was determined with an exact test using the GENEPOP software (ver. 3.4) (http://genepop.curtin.edu.au).

**Table 1**
Primer sequences used in this study.

| Locus | Product length (bp) | Allele range | Primersequence | | Dye label | primer concentration (poml) | Reference |
|---|---|---|---|---|---|---|---|
| DXS7424 | 82–103 | 12–19 | F:AAACACAGGAAGACCCCATC | R:GGCTAAGAAGAATCCCGCACA | 6-FAM | 0.095 | [5] |
| GATA172D05 | 109–133 | 6–12 | F:TAGTGGTGATGGTTGCACAG | R:GATAATTGAAAGCCCGGATTC | 6-FAM | 0.380 | This study |
| HPRTB | 149–173 | 10–16 | F:TCTCTATTTCCATCTCTGTCTCC | R:GTCACCCCTGTCTATGGTCTCG | 6-FAM | 0.952 | This study |
| DXS8377 | 216–272 | 41–58 | F:CACTTCATGGCTTACCACAG | R:GACCTTTGGAAAGCTAGTGT | 6-FAM | 0.571 | [6] |
| GATA31E08 | 109–137 | 7–14 | F:TGTATAGACAGAGCTGGTGATG | R:GCTCACTTTTATGTGTGTATGTATCTCC | VIC | 0.077 | This study |
| DXS6810 | 140–168 | 14–21 | F:TGTCCTGCCCCAAAATATATG | R:GTGTTATCTACATGATAGGGA | VIC | 1.220 | This study |
| DXS7423 | 180–192 | 13–16 | F:GTCTTCCTGTCATCTCCCAAC | R:GTAGCTTAGCGCCTGGCACATA | VIC | 0.122 | This study |
| DXS981 | 211–234 | 10.3–18 | F:GTTTCCTCCTGCAAAATACAGC | R:GTCCAGCACCCAAGGAAGTC | VIC | 0.077 | This study |
| DXS6795 | 279–297 | 9–15 | F:TGTCTGCTAATGAATGATTTGG | R:GCCATCCCCTAAACCTCTCAT | VIC | 2.808 | This study |
| DXS6803 | 110–133 | 10–15.3 | F:GAAATGTGCTTTGACAGGAA | R:GCAAAAAGGGACATATGCTACTT | NED | 1.667 | This study |
| DXS6789 | 143–187 | 13–24 | F:TTGGTACTTAATAAACCCTCTTT | R:GTCCTATTGTATTAGTCAGG | NED | 1.667 | This study |
| DXS6800 | 195–219 | 16–22 | F:GTGTGAGTTTAATACTCCTTAAT | R:GCTCTTTATTTCTCAGACTGGC | NED | 1.667 | This study |
| DXS6809 | 232–280 | 26–38 | F:TGAACCTTCCTAGCTCAGGA | R:GTCTGGAGAATCCAATTTTGC | NED | 3.333 | This study |
| DXS7133 | 84–100 | 8–12 | F:AGCTTCCTTAGATGGCATTCA | R:GTTTTTAACGGTGTTCATGCTT | PET | 0.900 | [5] |
| DXS7132 | 131–155 | 11–17 | F:GAGCCCATTTTCATAATAAA | R:GCCAAACTCTATTAGTCAAC | PET | 5.400 | [6] |
| DXS9902 | 166–194 | 7–14 | F:GGTGAAGAGAAGCAGGAATT | R:GCAATACACATTCATATCAG | PET | 9.000 | This study |
| DXS101 | 200–230 | 21–31 | F:ACTCTAAATCAGTCCAAATATCT | R:AAATCACTCCATGGCACATGTAT | PET | 3.600 | [7] |
| DXS6807 | 251–275 | 11–17 | F:GAGCAATGATCTCATTTGCA | R:AAGTAAACATGTATAGGAAAAAGCT | PET | 3.600 | [8] |