Four PCR-based polymorphisms in the pseudoautosomal region of the human X and Y chromosomes

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Using clones or STS primers from the pseudoautosomal region we have identified 4 biallelic polymorphisms which can be typed by PCR and studied their allele frequencies using semen or buccal epithelial cells (1, 2). DXYS15, DXYS85, DXYS77 and the gene MIC2 have previously been genetically and/or physically mapped to the pseudoautosomal region of the human sex chromosomes (3–5). Pseudoautosomal inheritance for all 4 markers has been observed by single sperm typing (unpublished data).

DXYS15, DXYS85, MIC2. *Source/Description*: Polymorphisms were identified by comparing the DNA sequences of T-vector cloned PCR products (6) from 10 different individuals after amplification of the DXYS15 or DXYS85 pseudoautosomal STSs (4) and a portion of the MIC2 gene (pDP1002; 5, unpublished data). A single nucleotide variation (A→G) was identified in the STS for the DXYS15 locus, creating a *Fnu*4HI restriction enzyme cleavage site in one allelic form. A 4 bp deletion/insertion polymorphism was found at both DXYS85 and MIC2.

Primer Sequences for DXYS15:
077 5'-TAATACAAGCCAGACGAGCC-3'
088 5'-CACACATCACTGGAAATAGACTG-3'
Amplifies a 186 (uncut), 166 + 20 (cut) bp fragment.

Primer Sequences for DXYS85:
073 5'-TTTGCTGAGCACCTAGAAGG-3'
083 5'-TAGGTCCTCTAGGTGCAGGA-3'
Amplifies a 78/82 bp fragment.

Primer Sequences for MIC2:
061 5'-TCCCGTCCGTGGTGACAGGT-3'
062 5'-CAAGCAGAGCTTCCTGTTTCTCC-3'
Amplifies a 74/78 bp fragment.

Frequencies: Allele frequencies were estimated from genomic DNA of 56 (DXYS15), 55 (DXYS85) and 42 (MIC2) unrelated individuals. At each locus the alleles are about equal in frequency and the heterozygosity was estimated at 0.48 (DXYS15), 0.49 (DXYS85) and 0.49 (MIC2).

Protocol: Approximately 30 ng of DNA were amplified in a mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 1 unit Taq polymerase, 100 μM of each dNTP and 0.2 μM of each of the appropriate primers in a final volume of 50 μl. Forty cycles were carried out on a Perkin-Elmer 480 Thermocycler: 95°C for 45 sec, 60°C for 1 min, 72°C for 2 min; the final cycle was followed by a 5 min extension at 72°C and the first cycle was preceded by 10 min at 95°C. The allelic differences at DXYS85, DXYS15 and MIC2 were detected after electrophoresis in an 8% polyacrylamide gel in 1× TBE at 125 V for 1 h. For DXYS15, 10 μl of amplified DNA was digested with *Fnu*4HI.

DXYS77. *Source/Description*: This polymorphism was identified by amplifying a 800 bp subclone from phage λOX315 (for DXYS77, the human genomic insert of phage λOX315 overlaps λOX316, which has been mapped to the pseudoautosomal region of the human sex chromosomes (5, unpublished data)) and 6 unrelated human DNA samples and examining them using Denaturing Gradient Gel Electrophoresis (7). Double stranded sequencing after cloning the PCR products revealed a biallelic nucleotide variation (G→A) at a site called PA1. The alleles can be distinguished by *Sty*I digestion (allele 1: 800 bp, allele 2: 500 + 300 bp).

Primer Sequences for DXYS77:
NA 125 5'-TATGGATCCTTCAGGAGCATCCCATGAAC-3'
NA 126 5'-TATGGATCCGCAAACCATATGGATTCTGC-3'
Both primers contain a *Bam*HI site at their 3'-ends to facilitate cloning of PCR products and amplify a 800 bp segment.

Frequencies: Allele frequencies for PA1 were estimated from genomic DNA of 31 unrelated individuals. The heterozygosity was estimated at 0.10 (frequencies: allele 1 — 0.95, allele 2 — 0.05).

Protocol: PCR of approximately 50 ng DNA was carried out as above except that 2 units Taq polymerase, 188 μM of each dNTP and 1 μM of primers in a final volume of 100 μl were used. Twenty-five cycles were carried out (94°C for 1 min, 62°C for 1 min, 72°C for 10 min, with each extension at 72°C being 30 sec longer after every cycle; the first cycle was preceded by 10 min at 95°C). Seventeen μl of amplified DNA after Centricon-30 filtration were digested with *Sty*I. The restriction fragments were resolved by electrophoresis in a 2% agarose gel.

Other Comments: A second polymorphism (called PA2) at DXYS77 and separated from PA1 by 57 bp has also been identified. This C→T transition can be detected by introducing a *Cla*I restriction site in PCR products from alleles containing T in order to distinguish the two alleles (unpublished data).

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