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**FOR THE RECORD**

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# 6 Y-SNP Typing of China and Korean Samples Using Primer Extension and DHPLC

**POPULATION:** Chinese, Korean.

**KEYWORDS:** forensic science, Y-chromosomal SNP, M9, M35, M96, M98, M102, M165, population genetics, single-base extension, Chinese Han population

TABLE 1—*SNPs investigated in this study.*

| SNP | Polymorphism | Fw/Rv Primer 5′–3′ | Amplicon Size (bp) |
|---|---|---|---|
| M35 | G/C | GAAACTGAGAGGGCATGGTC<br>GGAGCTGTGGTGAATGAACA | 130 |
| M165 | G/C | GATGACAGAATGCGTTCACCTTT<br>ATGTGTAAATATTTCAGGTAAAACCACTCT | 148 |
| M102 | G/C | GAAATTTATTAAATGAAGTATAGATTGAATACAAG<br>TCCTTAATCTCTAGGGGTTTTACAAA() | 210 |
| M9 | C/G | CGCTGCAGCATATAAAACTTTC<br>TGAAGCTCGTGAAACAGATTAGA | 233 |
| M98/M96 | G/C | AGATTCACCCACCCACTTTG<br>GAGCCTCAGGATTCAAAGGA | 396 |

Blood specimens of unrelated individuals were obtained from 105 males of a Chinese Han ethnic group (Chengdu, China) and 95 males of a Korean group (Seoul). DNA was extracted using the Chelex100 method (1).

Two multiplex single-base primer extension sets were used for the six Y-chromosome biallelic makers (multiplexI: M9, M35, M98, multiplexII: M96, M102, M165). The sequence of each locus was obtained from GenBank (http://www.ncbi.nlm.nih.gov) using a nucleotide basic local alignment search tool. Published PCR primers were initially used as the reference sequence for each Y SNP locus, but all of them needed to be redesigned with the Primer 3.0 program v. 0.2 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). All primers were selected to have theoretical melting temperatures between 58°C and 63°C, with maximum Tm differences of 2°C between the whole set of primers. Primer length was chosen between 19 and 30 nucleotides to provide good specificity for single target sequences. Each primer pair was tested for primer–primer interactions, and the primer sequences were checked to avoid similarities with repetitive sequences or with other loci in the genome. Table 1 shows the sequences of the amplification primers redesigned.

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The extension primers were designed so that they were well spaced from one other in the denaturing high-performance liquid chromatography (DHPLC), and the maximum length of the extension products was 45 nucleotides for M165 (Table 2). Extension primers were selected to have a predicted annealing temperature of *c*. 60°C and were screened for hairpin and primer dimer interactions using an in-house primer-checking program (2). Primer concentrations were adjusted in order to obtain sufficient RFU peak heights.

SNP genotyping was accomplished by multiplex polymerase chain reaction (PCR) with primers amplifying fragments between 130 and 396 nucleotides, multiplex primer extension, and DHPLC analysis of extension products.

Multiplex PCR: 1 ng of DNA was submitted to amplification in a GeneAmp PCR System 9600 (Perkin-Elmer, Applied Biosystems, Foster City, CA) thermal cycler in a final volume of 37.5 μL, composed of 10 × PCR reaction buffer, 200 μM dNTPs, 2.5 mM $MgCl_2$, 1.5 U Taq polymerase (MBI) and primers (0.25 μM for M35; 0.15 μM for M165; 0.30 μM for M102; 0.3 μM for M9; and 0.3 μM for M96/98). The thermal cycling program was carried out using the following conditions: 94°C for 3 min; 32 cycles of (94°C for 30 sec, 61°C for 30 sec, 72°C for 30 sec); 72°C for 7 min; 4°C until removal from the thermocycler. The PCR products were analyzed by horizontal nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system and visualized by silver staining (3).

TABLE 2—*Extension primers used in this study.*

| SNP | Primer Sequence 5′–3′ | Length (nt) | Concentration (μM) |
|---|---|---|---|
| MultiplexI | | | |
| M35 | $(T)_3$CAATTTTCCTTTGGGACACTG | 24 | 0.2 |
| M9 | $(T)_6$AACGGCCTAAGATGGTTGAAT | 27 | 0.2 |
| M98 | TCCCACGGGTAATTAACACTG | 21 | 0.2 |
| MultiplexII | | | |
| M96 | $(T)_3$AACTTGGAAAACAGGTCTCTCATAATA | 30 | 0.3 |
| M165 | $(T)_5$CCACTCTATTAGTATACCACTAATTCAATT | 35 | 0.4 |
| M102 | $(GACT)_2$GCTGTTTATTCTTATTGTCTTTTCACATCTTA | 40 | 0.35 |

TABLE 3—*Allele frequency of six Y-SNP loci in a Chinese population and a Korean population.*

| SNP Name | Polymorphism | China ($N = 105$) | Korean ($N = 95$) |
|---|---|---|---|
| M35 | G/C | 0.981/0.019 | 0.970/0.030 |
| M9 | G/C | 0.582/0.418 | 0.724/0.276 |
| M98 | G/C | 1.000/0.000 | 0.991/0.009 |
| M96 | G/C | 1.000/0.000 | 1.000/0.000 |
| M165 | G/C | 0.991/0.009 | 0.970/0.030 |
| M102 | G/C | 0.991/0.009 | 1.000/0.000 |

Two multiplex primer extension reactions were carried out in a total volume of 20 μL using 3.0 U of VentR®(exo-) Taq DNA, 2.0 μL of 10 × Thermol Reaction Buffer (NEB), 3.0 μL of PCR purified template, 0.8 μL of ddGTP(200 μM), and 1 μL of a stock solution of extension primers to produce the final primer concentrations indicated in Table 2. Extension reactions were incubated as follows: 95°C for 5 min, 45 cycles of 95°C for 30 sec, 50°C for 40 sec, and 65°C for 2 min.

Allelic frequencies were calculated through the gene-counting method, and gene diversity was estimated according to Hou's method (4). The allelic frequencies and haplotype distribution of the six SNP in the two ethnic groups studied are given in Table 3.

The complete data can be accessed at shimeisen2000@yahoo.com.cn

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