

HABC Genotype Data Submission Form

Submitting Investigator																	
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Date Submitted	5/27/2005																
Polymorphism Information																	
Name	SIM1 Ala371Val polymorphism																
RS# or Unique Identifier	rs3734355																
Gene	Single-minded 1 (<i>SIMI</i>)																
Chromosome position	chr6:100975442																
Alleles	G/A																
Assay Information																	
Genotyping Method	Template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI). Reference: Hsu TM, et al. <i>Biotechniques</i> . 2001 Sep;31(3):560-8																
Genotypes in HWE (Y/N) (attach HWE Form)	Yes. Caucasian genotypes were 1288: 399: 38 (GG: GA: AA). African American genotypes were 1133: 73: 2.																
Amount of DNA used	5 ng/assay																
PCR primers	rs3734355 P1: GGAGGCAGATAGTTTAgAGAACC rs3734355 P2: TCCTATACCAGCAGCTCCAC																
PCR Components and Concentrations (Taq, buffer, MgCl ₂ , primers, DMSO, other reagents)	<table style="width: 100%; border: none;"> <tbody> <tr> <td style="width: 50%;">10 x Platinum Taq buffer (Invitrogen)</td> <td style="width: 50%;">0.5 µL</td> </tr> <tr> <td>50 mM MgCl₂</td> <td>0.25 µL</td> </tr> <tr> <td>10 mM dNTPs</td> <td>0.25 µL</td> </tr> <tr> <td>Primers (10 mM)</td> <td>0.012 µL each</td> </tr> <tr> <td>Platinum Taq (Invitrogen)</td> <td>0.02 µL</td> </tr> <tr> <td>DMSO</td> <td>0.25 µL</td> </tr> <tr> <td>ddH₂O</td> <td>4.706 µL</td> </tr> <tr> <td>TOTAL</td> <td>6 µL</td> </tr> </tbody> </table>	10 x Platinum Taq buffer (Invitrogen)	0.5 µL	50 mM MgCl ₂	0.25 µL	10 mM dNTPs	0.25 µL	Primers (10 mM)	0.012 µL each	Platinum Taq (Invitrogen)	0.02 µL	DMSO	0.25 µL	ddH ₂ O	4.706 µL	TOTAL	6 µL
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<p>PCR Cycling conditions (time and temperature for each step, # of cycles, etc.)</p>	<p>PCR program:</p> <p>SNPPCR45 95 for 2:00min 92 for 10s 58 for 20s 68 for 30s GoTo 2 44 Times 68 for 10:00min 4 Forever end</p> <p>PCR cleanup program (SAP)</p> <p>37 for 45min 90 for 15min 4 forever end</p> <p>TDI program: for 20 cycles which is standard. For some assays we may use more or less cycles, between 5 and 80, for optimum result</p> <p>TDI_20 95 for 2.00min 95 for 15s 55 for 30s Go to 2 19 times 4 forever end</p>
<p>Detection Oligo(s) (if applicable)</p>	<p>rs3734355 P3: GCTGGAGAGCCGGGATTTG</p>
<p>Other Reaction Conditions (detection reaction components, incubation conditions, gel %, etc.)</p>	<p>The genotyping method was FP-TDI. After the PCR, the reaction was treated with shrimp alkaline phosphatase (SAP) for 90 minutes at 37°C. Subsequently, an appropriate dye terminator reaction was then initiated, containing 2 µL of 10 x reaction buffer, 1 µL of the dye terminators (in this case, C/T), 0.05 µL of the detection oligo (10 mM), 0.05 µL of Acyclopol, and 9.9 µL of ddH₂O. The samples were subjected to the extension reaction (TDI program as noted above) and genotypes were read in a plate reader.</p>
<p>Other Assay Info</p>	