Appendix 4: PCR conditions for African-American proband variant screening

Reaction condition	PCR reagents*	Cycling conditions
1 (standard)	*Qiagen HotStarTaq, 1.5 mM Mg:* 10X PCR buffer 2.5 µL 2 mM dNTPs 2.5 µL Q solution 5.0 µL F primer 1.0 µL R primer 1.0 µL Taq (5U/µL) 0.1 µL d.H_2O 9.9 µL 22 µL per well	*PCR cycle:* 1 cycle: 95°C for 5 min 10 cycles: 95°C for 30 sec 66°C for 30 sec (-1°C/cycle) 72°C for 30 sec 40 cycles: 95°C for 30 sec 56°C for 30 sec 72°C for 30 sec 1 cycle: 72°C for 10 min Hold: 4°C
2	*Qiagen HotStarTaq, 1.75 mM Mg:* 10X PCR buffer 2.5 µL 2 mM dNTPs 2.5 µL Q solution 5.0 µL $MgCl_2$ 0.25 µL F primer 1.0 µL R primer 1.0 µL Taq (5U/µL) 0.1 µL d.H_2O 9.65 µL 22 µL per well	Same as above
3	*Qiagen HotStarTaq, 2.25 mM Mg:* 10X PCR buffer 2.5 µL 2 mM dNTPs 2.5 µL Q solution 5.0 µL $MgCl_2$ 0.75 µL F primer 1.0 µL R primer 1.0 µL Taq (5U/µL) 0.1 µL d.H_2O 9.15 µL 22 µL per well	Same as above
4	*Invitrogen Platinum Taq, 1.5 mM Mg:* 10X PCR buffer 2.5 µL 2 mM dNTPs 2.5 µL $MgCl_2$ 0.75 µL 5M betaine 2.5 µL F primer 1.0 µL R primer 1.0 µL Taq (5U/µL) 0.2 µL d.H_2O 11.55 µL 22 µL per well	Same as above
5	*Qiagen HotStarTaq, 1.5 mM Mg, no Q:* 10X PCR buffer 2.5 µL 2 mM dNTPs 2.5 µL F primer 1.0 µL R primer 1.0 µL Taq (5U/µL) 0.1 µL d.H_2O 14.9 µL 22 µL per well	Same as above

* All reaction conditions use 3 µL of genomic DNA at 10 ng/µL; this brings all final reaction volumes up to 25 µL.