

A) Combined MasterMix and Reporter Mix

Final Volume (Multiple	rhAMP master Mix	1000ul	Vol
	rh Reporter Mix with 50ul	100	5 ul

B) Samples and Primers

	Reporter	Quencher	Task
Allele1 (Meth)	FAM	NFQ-MGB	1/1
Allele2 (Unmeth)	VIC	NFQ-MGB	2/2

Primers

Primer Name	ID
W1_2554	CD.GT.J8HB3172.1
W3_0222	CD.GT.JYVK6101.1
S1_1033	CD.GT.SPDR8897.10
S3_1292	CD.GT.GYJV9231.1
G1_1884	CD.GT.WFVP0601.1
G3_0126	CD.GT.PVWC1084.1

C) Working Mix

	Samples	W1_2554	W3_0222	S1_1033	S3_1292	G1_1884	G3_0126
	5ul	6	6	6	6	6	6
	Final	Samples	# of Samples	# of Samples	# of Samples	# of Samples	# of Samples
Master Mix + Reporter	2.65	15.9	15.9	15.9	15.9	15.9	15.9
SNP assay (20X)	0.25	1.5	1.5	1.5	1.5	1.5	1.5
Water	1.1	6.6	6.6	6.6	6.6	6.6	6.6
Mix Volume	4	24	24	24	24	24	24

Sample	1	1
Final Volume	5	5

D) qPCR Template

	1	2	3	4	5	6	7	8	9	10	11	12
A				W1_2554 NTC	W3_0222 NTC	S1_1033 NTC	S3_1292 NTC	G1_1884 NTC	G3_0126 NTC			
B				W1_2554 NTC	W3_0222 NTC	S1_1033 NTC	S3_1292 NTC	G1_1884 NTC	G3_0126 NTC			
C				W1_2554 Sample	W3_0222 Sample	S1_1033 Sample	S3_1292 Sample	G1_1884 Sample	G3_0126 Sample			
D				W1_2554 Sample	W3_0222 Sample	S1_1033 Sample	S3_1292 Sample	G1_1884 Sample	G3_0126 Sample			
E				W1_2554 gBlock_W1_U	W3_0222 gBlock_W3_U	S1_1033 gBlock_S1_U	S3_1292 gBlock_S3_U	G1_1884 gBlock_G1_U	G3_0126 gBlock_G3_U			
F				W1_2554 gBlock_W1_U	W3_0222 gBlock_W3_U	S1_1033 gBlock_S1_U	S3_1292 gBlock_S3_U	G1_1884 gBlock_G1_U	G3_0126 gBlock_G3_U			
G				W1_2554 gBlock_W1_M	W3_0222 gBlock_W3_M	S1_1033 gBlock_S1_M	S3_1292 gBlock_S3_M	G1_1884 gBlock_G1_M	G3_0126 gBlock_G3_M			
H				W1_2554 gBlock_W1_M	W3_0222 gBlock_W3_M	S1_1033 gBlock_S1_M	S3_1292 gBlock_S3_M	G1_1884 gBlock_G1_M	G3_0126 gBlock_G3_M			

E) Thermal Cycling

Step	Temperature (°C)	Time (min:sec)	Cycles
Enzyme Activation	95	10:00	1
Denature	95	0:10	40
Anneal	60	0:30	
Extend	68	0:45	
PCR- read	60	0:30	1