EpiGe qPCR Template

A) Combined MasterMix and Reporter Mix

				Vol
Final Volume (Multiples of 50ul)	rhAMP master Mix	1000ul	**150**	ul
	rh Reporter Mix with Dye	50ul	7.5	ul

B) Samples and Primers

Primers

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

C) Working Mix

	Samples	cg18849583	cg01268345	cg10333416	cg12925355	cg25542041	cg02227036
	5ul	8	8	8	8	8	8
	Final	# of Samples	# of Samples	# of Samples	# of Samples	Samples	# of Samples
Master Mix + Reporte	2.65	23.85	23.85	23.85	23.85	23.85	23.85
SNP assay (20X)	0.25	2.25	2.25	2.25	2.25	2.25	2.25
Water	1.1	9.9	9.9	9.9	9.9	9.9	9.9
Mix Volume	4	36	36	36	36	36	36

Sample	1	1
Final Volume	5	5

D) qPCR Template

	1	2	3	4	5	6	7	8	9	10	11	12
A				cg18849583 NTC	cg01268345 NTC	cg10333416 NTC	cg12925355 NTC	cg25542041 NTC	cg02227036 NTC			
B				cg18849583 NTC	cg01268345 NTC	cg10333416 NTC	cg12925355 NTC	cg25542041 NTC	cg02227036 NTC			
C				cg18849583 Sample	cg01268345 Sample	cg10333416 Sample	cg12925355 Sample	cg25542041 Sample	cg02227036 Sample			
D				cg18849583 Sample	cg01268345 Sample	cg10333416 Sample	cg12925355 Sample	cg25542041 Sample	cg02227036 Sample			
E				cg18849583 gBlock_Unmethylated	cg01268345 gBlock_Unmethylated	cg10333416 gBlock_Unmethylated	cg12925355 gBlock_Unmethylated	cg25542041 gBlock_Unmethylated	cg02227036 gBlock_Unmethylated			
F				cg18849583 gBlock_Unmethylated	cg01268345 gBlock_Unmethylated	cg10333416 gBlock_Unmethylated	cg12925355 gBlock_Unmethylated	cg25542041 gBlock_Unmethylated	cg02227036 gBlock_Unmethylated			
G				cg18849583 gBlock_Methylated	cg01268345 gBlock_Methylated	cg10333416 gBlock_Methylated	cg12925355 gBlock_Methylated	cg25542041 gBlock_Methylated	cg02227036 gBlock_Methylated			
H				cg18849583 gBlock_Methylated	cg01268345 gBlock_Methylated	cg10333416 gBlock_Methylated	cg12925355 gBlock_Methylated	cg25542041 gBlock_Methylated	cg02227036 gBlock_Methylated			

E) Thermal Cycling

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