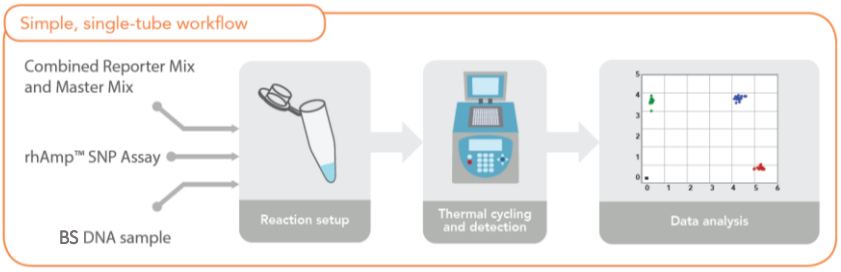
EpiGe Protocol

Adapted EpiGe Protocol to the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System.

Material



- 6 rhAmp assays (IDT) for each one of the 6 cytosines of the EpiWNT-SHH panel:
  - cg18849583
  - cg01268345
  - cg10333416
  - cg12925355
  - cg25542041
  - cg02227036

- 12 Synthetic Controls (gBlocks): Two controls (Methylated and Unmethylated) for each cytosine of the EpiWNT-SHH panel.

- rhAmp® rhAmp Genotyping Master Mix.

- rhAmp® Reporter Mix w/Reference. Use the Reporter Mix with or without reference dye, as indicated in the table below or by checking with the manufacturer of your instrument:

PCR system	Reference dye required	
	Yes	No
7900HT Fast Real-Time PCR System (Thermo Fisher Scientific)	X	
StepOne™ and StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific)	X	
Mx3005P™ and Mx4000P™ qPCR System (Agilent)	X	
7500 Real-Time PCR System (Thermo Fisher Scientific)	X	
Viia™7 Real-Time PCR System (Thermo Fisher Scientific)	X	
QuantStudio™ Flex Systems (Thermo Fisher Scientific)	X	
Biomark™ HD (Fluidigm)	X	
CFX, iQ™, and Opticon™ Real-Time PCR Detection Systems (Bio-Rad)		X
LightCycler® Real-Time PCR Systems (Roche)		X

* For instruments not listed, please check with the manufacturer.

Method

1. Use the template “EpiGe_Genotyping_Template.xlsx” to prepare the working Master mix combining the Reporter Mix with Dye and the rhAmp Master Mix in a 1:20 proportion. Take into account that all analysed samples should be replicated at least 2 times within each experiment. EpiGe-App only accepts qPCR results in which one sample has been analysed at the time and have at least 2 NTC replicates for each assay.
2. To a new tube or vial, add the following:

	Volume
rhAMP master Mix	**150 ul**
rhAMP Reporter Mix with Dye	7.5ul

3. Mix and vortex.
4. Prepare the six EpiGe SNP Genotyping Assay Reaction Mixes (Combined Master Mix, Reporter Mix, and EpiGe assay) to a final volume of 5µl for 9 samples (2 Methylated DNA synthetic control (gBlock, 2 Unmethylated DNA synthetic control (gBlock), 2 NTC, 2 Samples and 1 additional reaction to account for pipetting errors).

	Samples	cg18849583	cg01268345	cg10333416	cg12925355	cg25542041	cg02227036
	5ul Final	8 # of Samples	8 # of Samples	8 # of Samples	8 # of Samples	8 Samples	8 # 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

5. Vortex and briefly centrifuge before use.
6. Add 4µl of EpiGe Master Mix to each well of the qPCR plate or strip and 1µl of the bisulfite converted sample or DNA synthetic control (gBlock).

	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			