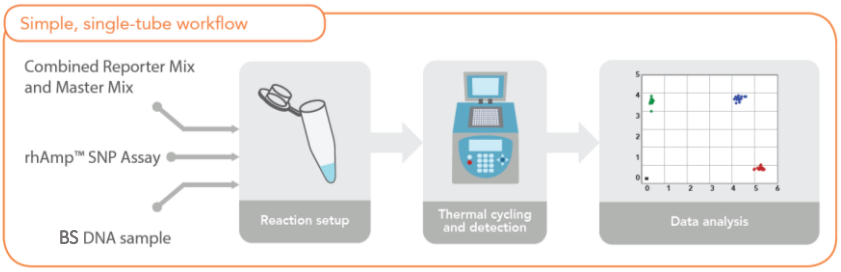
# EpiGe Protocol

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.

| Cytosine | rhAmp_ID |
|---|---|
| S1_1033 | CD.GT.SPDR8897.10 |
| S3_1292 | CD.GT.GYJV9231.1 |
| W1_2554 | CD.GT.JBHB3172.1 |
| W3_0222 | CD.GT.JYVK6101.1 |
| G1_1884 | CD.GT.WFVP0601.1 |
| G3_0126 | CD.GT.PVWC1084.1 |

- 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* | **100 ul** |
| *rhAMP Reporter Mix with Dye* | 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 6 samples (2 NTC, 2 Samples and 2 additional reactions to account for pipetting errors).

| | **Samples** | W1_2554 | W3_0222 | S1_1033 | S3_1292 | G1_1884 | G3_0126 |
|---|---|---|---|---|---|---|---|
| | 5ul Final | 6 Samples | 6 # of Samples | 6 # of Samples | 6 # of Samples | 6 # of Samples | 6 # 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 |

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 | | | | 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 | | | |