FOR RESEARCH USE ONLY

Use this protocol to determine whether your DNA samples are candidates for either the Infinium HD FFPE genotyping or Infinium HD FFPE methylation assay. Figure 1 illustrates the correct workflows for both Infinium HD FFPE assays. Examine the diagram for reference to the required Illumina protocols and materials.

Prior to running the Infinium HD FFPE QC assay extract DNA from FFPE samples using your preferable extraction protocol. Determine DNA concentration in your samples using PicoGreen (recommended by Illumina) or similar fluorescent dye assays. Please note that measurement of optical density of DNA samples at 260 nm (OD 260) usually overestimates the DNA concentration in samples extracted from FFPE tissues.

Analyze each purified DNA sample in 3 replicates. To perform Infinium HD FFPE QC assay you have to purchase 2XqPCR master mix containing green fluorescent dye and PCR plates compatible with your qPCR instrument from authorized vendors. This protocol is to be used in conjunction with the Infinium HD FFPE QC Kit (WG-321-1001).

If you use a standard 384 well plate or an Eco 48 well plate, keep the final reaction volume at 10 µl. If you use a 96 well plate, keep the final reaction volume at 20 µl.

Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage</th>
<th>Supplied By</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x qPCR Master Mix containing green fluorescent dye</td>
<td>Depends on number of DNA samples</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>DNA samples</td>
<td>Determined by User</td>
<td>-20°C</td>
<td>User</td>
</tr>
<tr>
<td>qPCR plate(s)</td>
<td>Depends on number of DNA samples</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>DiH₂O</td>
<td>Depends on number of DNA samples</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>QC Primer (QCP) Reagent</td>
<td>0.39 ml</td>
<td>-20°C</td>
<td>Illumina</td>
</tr>
<tr>
<td>QC Template (QCT) Reagent</td>
<td>0.22 ml</td>
<td>-20°C</td>
<td>Illumina</td>
</tr>
</tbody>
</table>

Figure 1  FFPE Workflow
**Preparation**
- Measure the concentration of DNA extracted from FFPE samples using PicoGreen or similar fluorescent dye assay and dilute the DNA to a concentration of 1 ng/µl.
- Thaw the QCP and QCT reagent tubes to room temperature and vortex to mix the contents.

Note: If you plan to use QCT for multiple PCR runs, create 10 µl aliquots of QCT in tubes labeled "QCT_ST" and keep them frozen at -20°C. Use a fresh QCT aliquot for each PCR run. The original box can accommodate at least 6 aliquots.
- Thaw the 2x qPCR Master Mix tube(s). The number of tubes will be determined by the number of samples you are analyzing.

**Steps**
1. Take a fresh 10 µl aliquot of QCT and dispense 990 µl of DiH$_2$O water to it to create a 100-fold dilution of QCT.
2. Mix by brief vortexing and collect the droplets with a brief spin at 280 xg.
3. For 10 µl reaction volumes:
   a. Pipette 2 µl of the 100-fold diluted QCT from the QCT-ST tube in 3 wells of the qPCR plate.
   b. Pipette 2 µl of genomic DNA (1 ng/µl) from FFPE samples into 3 wells of the qPCR plate.
   c. Pipette 2 µl of DiH$_2$O water in 3 wells of the qPCR plate for "no template control" (NTC).
4. For 20 µl reaction volumes:
   a. Pipette 4 µl of the 100 fold dilution of QCT from the QCT-ST tube in 3 wells of the qPCR plate.
   b. Pipette 4 µl of genomic DNA into 3 wells of the qPCR plate.
   c. Pipette 4 µl of DiH$_2$O water in 3 wells of the qPCR plate for "no template control" (NTC).
5. Prepare the qPCR premix using the following table. Volumes are per well of DNA sample (or control) you are analyzing. Example: If you are analyzing 100 DNA samples in a 384 well plate, you would prepare the qPCR premix for 337 replicates: (306 replicates + 10% overfill). The final volume would be 337 x 8 µl of prepared qPCR premix = 2.696 ml.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C*</td>
<td>2 minutes</td>
</tr>
<tr>
<td>95°C*</td>
<td>10 minutes</td>
</tr>
</tbody>
</table>

**X 40**
- 95°C 30 seconds
- 57°C 30 seconds
- 72°C 30 seconds

*If required by the Master Mix manufacturer.

**Analyzing Data**
In accordance with the MIQE Guidelines for Real-Time PCR experiments, $C_t$ (threshold cycle) will be referred to as $C_q$ (quantification cycle) as a quantification value.
1. Check the NTC wells for any amplification. There should be zero to very inefficient amplification. Data is acceptable if the amplification in NTC samples is >10 cycles after QCT_ST samples.
2. Ensure that there is good amplification for all replicates and remove any replicate $C_q$ values that diverge by more than half a unit.
3. Obtain $C_q$ values for all wells and compute average $C_q$ values for each FFPE and QCT_ST sample.
4. Subtract the average $C_q$ value for QCT_ST from the average $C_q$ value for each sample to compute the Delta $C_q$ value for each sample.
5. All samples with Delta $C_q$ value below 5 can be selected for use with the Infinium HD FFPE Assay and the Infinium HD FFPE Methylation Assay.
6. Proceed to the Infinium HD FFPE Restore Protocol (Part # 15020981 Rev. C) with your DNA samples and use the Infinium HD FFPE QC Kit (WG-321-1001) to restore your DNA samples.

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