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Infinium HD FFPE QC Assay Protocol

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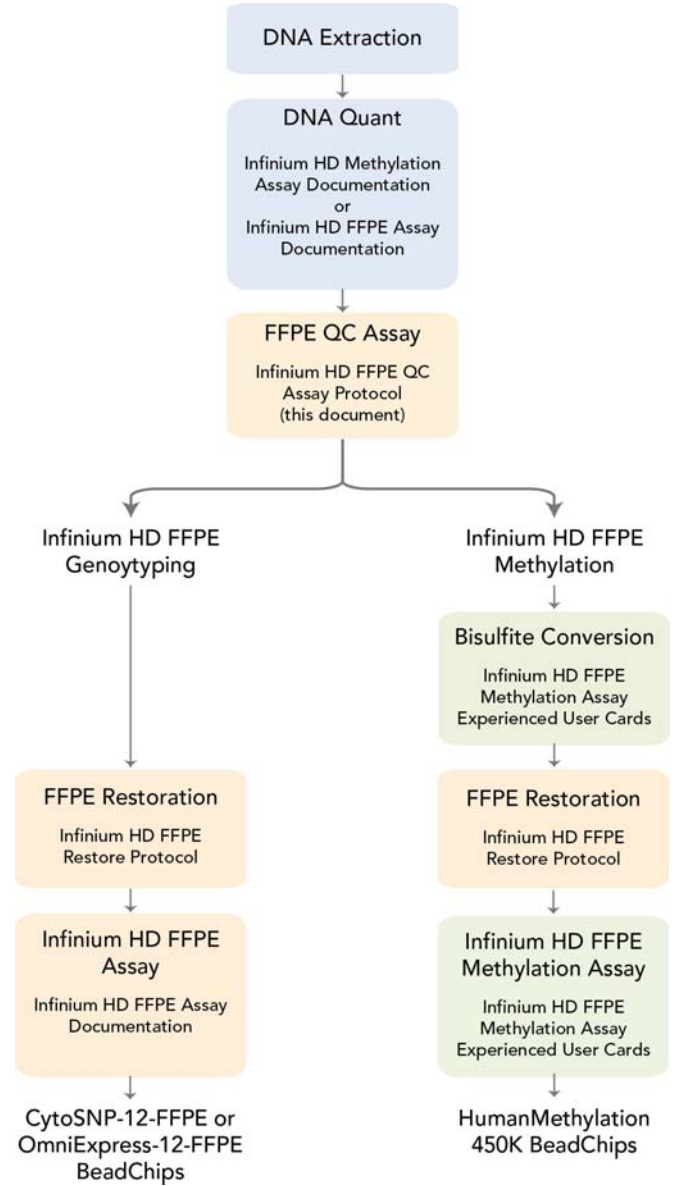
Analyze each purified DNA sample in 3 replicates. To perform Infinium HD FFPE QC assay you have to purchase 2XqPCR master mix containing green fluorescent dye and PCR plates compatible with your qPCR instrument from authorized vendors. This protocol is to be used in conjunction with the Infinium HD FFPE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

Item	Quantity	Storage	Supplied By
2x qPCR Master Mix containing green fluorescent dye	Depends on number of DNA samples		User
DNA samples	Determined by User	-20°C	User
qPCR plate(s)	Depends on number of DNA samples		User
DiH$_2$O	Depends on number of DNA samples		User
QC Primer (QCP) Reagent	0.39 ml	-20°C	Illumina
QC Template (QCT) Reagent	0.22 ml	-20°C	Illumina

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.

	10 µl reaction volumes	20 µl reaction volumes
2x qPCR Master Mix	5 µl	10 µl
QCP	1 µl	2 µl
DiH$_2$O	2 µl	4 µl
Total volume added per well	8µl	16µl

6   Mix the qPCR premix container by inverting 10 times and tap container on lab bench to collect the droplets.

7   Transfer the qPCR premix into a clean trough.

8   Using a multi-channel pipette (optional) dispense 8 µl (for 10 µl final volume) or 16 µl (for 20 µl final volume) of the qPCR premix into each well containing gDNA, QCT, and NTC.

Note: Take care to pipette accurately into the wells, as variations in volume will affect the assay. Change tips after each pipetting step.

9   Seal the plate according to the manufacturer's instructions and briefly spin at 280 xg.

10   Make sure that the optical seal is clean from any liquid or dust. Place the plate in the qPCR machine in the correct orientation.

11   Run the qPCR program using the following thermal profile:

	Temperature	Time
	50°C*	2 minutes
	95°C*	10 minutes
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 (threshhold 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.