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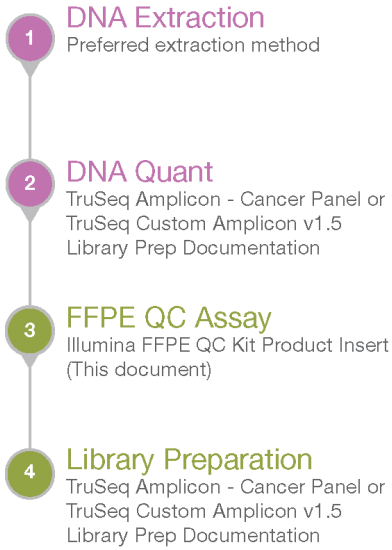
Illumina FFPE QC Kit for TruSeq Amplicon - Cancer Panel and TruSeq Custom Amplicon v1.5

For Research Use Only. Not for use in diagnostic procedures.

This protocol outlines a simple qPCR-based quality control method for FFPE DNA that predicts assay success for the TruSeq® Amplicon - Cancer Panel and TruSeq Custom Amplicon. This protocol is not required for high-quality DNA.

- Before running the Illumina® FFPE QC assay, extract DNA from FFPE tissue samples using your preferred extraction protocol. Determine DNA concentration of the samples using PicoGreen or similar fluorescent dye assays. Measurement of optical density at 260 nm (OD 260) is usually not accurate for DNA extracted from FFPE tissues.
- Analyze each purified DNA sample in 3 replicates.
- To perform the Illumina FFPE QC assay, you need 2x qPCR master mix containing green fluorescent dye and PCR plates compatible with your qPCR instrument from authorized vendors. The Illumina Eco system is recommended. This protocol is for use with the Illumina 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.

Item	Quantity	Storage	Supplied By
2x qPCR Master Mix containing green fluorescent dye	Depends on number of DNA samples	As listed by MFR	User
DNA samples	Determined by User	-20°C	User
qPCR plates	Depends on number of DNA samples	Room temperature	User
DI water	Depends on number of DNA samples	Room temperature	User
QC Primer (QCP) Reagent	0.39 ml	-20°C	Illumina
QC Template (QCT) Reagent	0.22 ml	-20°C	Illumina



Preparation

1. Measure the concentration of DNA extracted from FFPE samples using PicoGreen or similar fluorescent dye assay.
2. Dilute the DNA to a concentration of 1 ng/µl.
3. Thaw the QCP and QCT reagent tubes to room temperature. 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.
4. Thaw the 2x qPCR Master Mix tubes. The number of tubes needed depends on the number of samples you are analyzing.

Procedure

1. Create a 100-fold dilution of QCT by mixing a fresh 10 µl aliquot of QCT and 990 µl of DI water.
2. Vortex briefly to mix. Centrifuge briefly at 280 × g to collect droplets.
3. For 10 µl reactions:
   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 DI water in 3 wells of the qPCR plate for "no template control" (NTC).

4 For 20 µl reactions:

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 DI water in 3 wells of the qPCR plate for "no template control" (NTC).

5 Prepare the qPCR premix using the following table. 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.

	For each 10 µl reaction	For each 20 µl reaction
2x qPCR Master Mix	5 µl	10 µl
QCP	1 µl	2 µl
DI water	2 µl	4 µl
Total volume added per well	8 µl	16 µl

6 Mix the qPCR premix by inverting 10 times. Tap tube 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 spin briefly 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
40 Cycles	95°C	30 seconds
	57°C	30 seconds
	72°C	30 seconds

* If required by the Master Mix manufacturer.

Data Analysis

In accordance with the MIQE Guidelines for Real-Time PCR experiments, Ct (threshold cycle) will be referred to as Cq (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 Cq values that diverge by more than half a unit.

3 Obtain Cq values for all wells and compute average Cq values for each FFPE and QCT_ST sample.

4 Subtract the average Cq value for QCT_ST from the average Cq value for each sample to compute the Δ Cq value for each sample.

5 All samples with Δ Cq value below or equal to 2 can be selected for use with the TruSeq Amplicon - Cancer Panel Library Preparation assay. Proceed to the *TruSeq Amplicon - Cancer Panel Library Preparation Guide* (Part # 15031875) with your DNA samples.

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