TruSeq FFPE DNA Library Prep QC Kit

Protocol Guide

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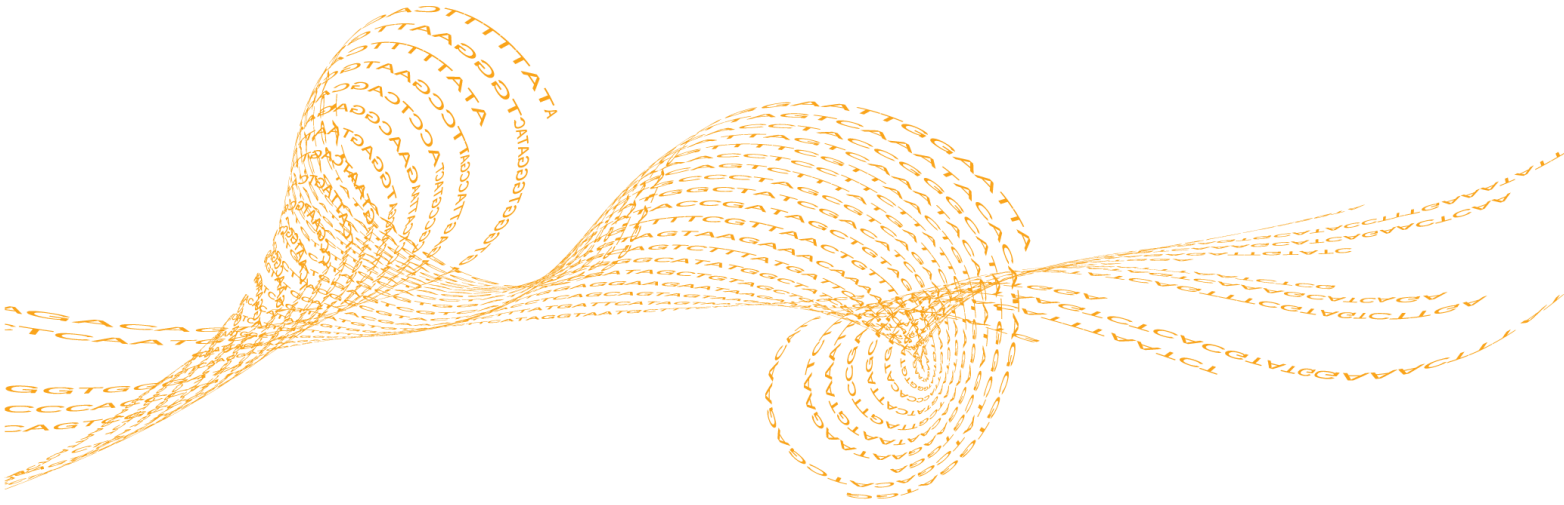

ILLUMINA PROPRIETARY
Catalog # FC-121-9016DOC
Material # 20001039
Document # 1000000002136 v00
October 2015

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Introduction

This protocol explains how to determine the fragmentation status and the amplification potential of formalin-fixed, paraffin-embedded (FFPE) extracted genomic DNA (gDNA) samples using the Illumina® TruSeq® FFPE DNA Library Prep QC Kit.

After DNA extraction, the FFPE QC Kit evaluates the quality of prospective DNA samples to determine if they are viable. The kit uses a real-time PCR assay that can be carried out using standard instrumentation and reagents purchased from an authorized vendor. The simple qPCR determines DNA quality and provides guidance on sequencing parameters, allowing researchers to assess sample quality before designing and running sequencing experiments. This step ensures that only samples that achieve the necessary sequencing quality are prepared, conserving resources that might be used on potentially low-quality, unrecoverable samples.

- Use the specified volumes and qPCR parameters following the instructions in the order listed.
- Review *Supporting Information* on page 9 before proceeding to confirm kit contents and make sure that you have the required equipment and consumables for the protocol.

Additional Resources

Visit the TruSeq FFPE DNA Library Prep QC Kit on the Illumina website for access to requirements and compatibility, frequently asked questions, and best practices.

DNA Input Recommendations

FFPE human tissues are a valuable source of material for molecular analysis and clinical applications. Several processes and protocols now exist for the extraction and purification of nucleic acids from FFPE samples. However, the quality of the extracted FFPE DNA is often compromised. For this reason, the DNA input and method used to prepare FFPE DNA libraries is critical to ensure high-quality deep whole-genome and amplicon sequencing.

FFPE gDNA input requirements for whole-genome and amplicon sequencing are based on the ΔCq obtained from this protocol. The quality of libraries and sequencing data depends on the quality of the gDNA extracted from FFPE samples. The higher the ΔCq of the samples, the lower the quality and higher the amount of input DNA required for deep whole human genome and amplicon sequencing.

See your respective library preparation reference guide or support page for specific DNA input requirements.

Quantify Input DNA

Use the following recommendations to quantify input DNA:

- Successful library preparation depends on accurate quantification of input DNA. To verify results, use multiple methods.
- Use fluorometric-based methods for quantification, such as Qubit or PicoGreen.
- DNA quantification methods that rely on intercalating fluorescent dyes measure only double-stranded DNA and are less subject to the presence of excess nucleic acids.
- Do not use spectrophotometric-based methods, such as NanoDrop, which measure the presence of nucleotides and can result in an inaccurate measurement of FFPE DNA.
- Quantification methods depend on accurate pipetting methods. Do not use pipettes at the extremes of volume specifications. Make sure that pipettes are calibrated.

Qualify FFPE gDNA

During this process, a qPCR reaction determines the fragmentation status and amplification potential of FFPE-extracted gDNA samples. The status and potential are compared to ACD1 control DNA to calculate a ΔCq value for each sample.

Consumables

- ACD1 (control DNA)
- QCP (QC Primer 204) (quality control primer)
- 1.7 ml microcentrifuge tubes
- 15 ml conical centrifuge tube
- FFPE gDNA samples
- KAPA SYBR FAST Universal 2X qPCR Master Mix
- Nuclease-free water
- One or both of the following:
  - Qubit dsDNA BR Assay Kit
  - Qubit dsDNA HS Assay Kit
- qPCR plate
- qPCR plate seal
- Qubit assay tubes or Axygen PCR-05-C tubes
- Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20

Preparation

The total volume required per reaction varies by qPCR plate or instrument:

- 20 µl reactions for 96-well plates
- 10 µl reactions for 384-well plates

1 Prepare the following consumables.

Reagent	Storage	Instructions
ACD1	-25°C to -15°C	Thaw at room temperature and then place on ice.
QCP	-25°C to -15°C	Thaw at room temperature and then place on ice.
KAPA SYBR FAST Universal 2X qPCR Master Mix	-25°C to -15°C	Thaw on ice and protect from light.

2 Remove FFPE gDNA from -25°C to -15°C storage and thaw at room temperature.

3 Prepare 10 ml Tris-HCl 10 mM, pH 8.5 with a final concentration of 0.1% Tween 20 in a 15 ml conical centrifuge tube.
This mixture is enough for 24 samples and is used for dilution and as a no template control (NTC).

4 Save the following FFPEQC program on the qPCR instrument:
- 95°C for 3 minutes
- 40 cycles of:
  - 95°C for 3 seconds
  - 66°C for 20 seconds
  - 72°C for 10 seconds

Quantify DNA

1. Flick the ACD1 and FFPE gDNA tubes to mix, and then centrifuge briefly. Do not vortex the tubes, which can further degrade the DNA.
2. Quantify ACD1 and FFPE gDNA using a fluorometric method, such as PicoGreen or Qubit dsDNA assays.

   NOTE
   Quantify ACD1 and FFPE gDNA in duplicate. If duplicates are not consistent, measure a third replicate. If you do not have enough FFPE gDNA to quantify in duplicate, a single quantification is possible. However, if the single quantification is inaccurate, then the input to both the qPCR assay and library prep might be incorrect.

   Qubit assays measure sample volumes from 1 µl to 20 µl. Add at least 2 µl each of ACD1 and FFPE gDNA to the tubes that contain the master mix. Choose the appropriate assay for your dsDNA sample concentration:
   - 100 pg/µl–1000 ng/µl concentration—Qubit dsDNA BR Assay Kit.
   - 10 pg/µl–100 ng/µl concentration—Qubit dsDNA HS Assay Kit.

   The ACD1 concentration is 10–50 ng/µl.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at 2°C to 8°C for up to 7 days.

Dilute and Requantify ACD1

1. Flick the ACD1 tube to mix, and then centrifuge briefly.
2. Dilute ACD1 to 2 ng/µl in Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Prepare enough volume for quantification and assay use.
3. Flick to mix, and then centrifuge briefly. Do not vortex.
4. In triplicate, quantify the diluted ACD1 using a Qubit dsDNA HS Assay Kit.
5. Based on the actual concentration, further dilute the ACD1 to 0.25 ng/µl in Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Prepare 30 µl.
6. Flick to mix, and then centrifuge briefly. Do not vortex.

Dilute and Requantify FFPE gDNA

1. Dilute FFPE gDNA to 2 ng/µl in Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Prepare enough volume for quantification and assay use.
2. Flick to mix, and then centrifuge briefly. Do not vortex.
3. In triplicate, quantify the diluted FFPE gDNA using a Qubit dsDNA HS Assay Kit.
4. Based on the actual concentration, further dilute the FFPE gDNA to 0.25 ng/µl in Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20. Prepare 30 µl.
5. Flick to mix, and then centrifuge briefly. Do not vortex.

Prepare Master Mix

1 Prepare a qPCR master mix in a sterile, nuclease-free microcentrifuge tube on ice. Make 10% excess.

NOTE
All samples, ACD1, and the No Template Control (NTC) are run in triplicate.

Reagent	Volume per 20 µl reaction	Volume per 10 µl reaction
QCP	1 µl	0.5 µl
KAPA SYBR Fast Master Mix Universal (2x)	10 µl	5 µl
Nuclease-free water	1 µl	0.5 µl

2 Pipette to mix, and then centrifuge briefly.

3 Place the tube on ice and protect it from light.

Prepare qPCR Plate

1 Determine the qPCR plate layout to run the FFPE gDNA samples, ACD1, and NTC in triplicate. For 24 samples, Illumina recommends the following plate layout.

Figure 1 Example: qPCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	ACD1	ACD1	ACD1	Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24
B	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 17	Sample 17	Sample 17			
C	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 18	Sample 18	Sample 18			
D	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 19	Sample 19	Sample 19			
E	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 20	Sample 20	Sample 20			
F	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 21	Sample 21	Sample 21			
G	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14	Sample 22	Sample 22	Sample 22			
H	Sample 7	Sample 7	Sample 7	Sample 15	Sample 15	Sample 15	Sample 23	Sample 23	Sample 23	NTC	NTC	NTC

2 Add the qPCR master mix to each well:
- For 20 µl total volume, add 12 µl.
- For 10 µl total volume, add 6 µl.

3 Add the following items and refer to Figure 1.
- For 20 µl total volume, add 8 µl of each item.
- For 10 µl total volume, add 4 µl of each item.

Item	Row	Column
Diluted ACD1	A	1, 2, 3
NTC	H	10, 11, 12
Each diluted FFPE gDNA sample (0.25 ng/µl)	Any 3 wells that do not contain ACD1 or NTC	

4 Pipette to mix.

5 Centrifuge at 280 × g for 1 minute.

Quantify by qPCR

1. Place the plate on the preprogrammed qPCR instrument and run the FFPEQC program.
2. Collect the Cq value of ACD1 and each FFPE gDNA sample.

   NOTE
   Make sure that there is good amplification of the ACD1 and remove outliers from a triplicate group that are > 0.5 Cq different from the rest of the group.

3. Using auto baseline correction, subtract the average Cq value of ACD1 from the average Cq value of each sample to yield the ΔCq value of each sample (Average Cq sample - Average Cq ACD1 = ΔCq sample).
4. Place ACD1, QCP, and FFPE gDNA in -25°C to -15°C storage.

Supporting Information

The protocols provided in this guide assume that you are familiar with the contents of this section and that you have the required equipment and consumables.

Acronyms

Acronym	Definition
ACD1	Amplicon Control DNA
FFPE	Formalin-fixed, paraffin-embedded
gDNA	genomic DNA
NTC	No template control
QC	Quality control
QCP	QC Primer 204 (quality control primer)

Kit Contents

Make sure that you have all the reagents before starting the protocol. The TruSeq FFPE DNA Library Prep QC Kit consists of 1 box. Store the components at -25°C to -15°C.

Kit Name	Catalog #
TruSeq FFPE DNA Library Prep QC Kit	FC-121-9999

Box 1, Store at -25°C to -15°C

Quantity	Reagent	Description
4	QCP	QC Primer 204
2	ACD1	Amplicon Control DNA

Consumables and Equipment

Make sure that you have the necessary user-supplied consumables and equipment before starting the TruSeq FFPE DNA Library Prep QC Kit protocol.

NOTE
The TruSeq FFPE DNA Library Prep QC Kit protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Consumables

Consumable	Supplier
1.7 ml microcentrifuge tubes	General lab supplier

Consumable	Supplier
15 ml conical tubes	General lab supplier
10 µl barrier pipette tips	General lab supplier
10 µl multichannel pipettes	General lab supplier
10 µl single channel pipettes	General lab supplier
20 µl barrier pipette tips	General lab supplier
20 µl multichannel pipettes	General lab supplier
20 µl single channel pipettes	General lab supplier
200 µl barrier pipette tips	General lab supplier
200 µl multichannel pipettes	General lab supplier
200 µl single channel pipettes	General lab supplier
KAPA SYBR FAST Universal 2X qPCR Master Mix	KAPA Biosystems, part # KK4602
Nuclease-free water	General lab supplier
qPCR plate	General lab supplier
qPCR plate seal	General lab supplier
Qubit assay tubes or Axygen PCR-05-C tubes	Life Technologies, catalog # Q32856 or VWR, part # 10011-830
One of the following: • Qubit dsDNA BR Assay Kit • Qubit dsDNA HS Assay Kit	Life Technologies, catalog # • Q32850 (BR 100 assays) • Q32853 (BR 500 assays) • Q32851
Tris-HCl 10 mM, pH 8.5	General lab supplier
Tween 20	Sigma-Aldrich, part # P7949

Equipment

Consumable	Supplier
Microplate centrifuge	General lab supplier
qPCR System	General lab supplier

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 2 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

Material # 20001039

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