Assessing FFPE DNA Quality: The "Illumina FFPE QC Kit" Enables Quantitation of Improvements in FFPE DNA Extraction Technologies

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Introduction

Formalin Fixation and Paraffin Embedding (FFPE) of tissues, a mainstay of clinical histological analysis, is rapidly being adopted for targeted, whole genome, and amplicon sequencing. The extreme formaldehyde fixation and tissue dehydration of FFPE-preserved tissue presents a technical challenge to reproducible DNA extraction of sufficient quality for Next Generation Sequencing (NGS).

In this technical note, we used the Illumina FFPE QC kit (2) to assess the quality of DNA extracted from two different extraction technologies using four different types of FFPE samples. The QC Kit assay is qPCR-based and compares the amplificability of the extracted DNA to a reference template. For the TruSeq® Amplicon - Cancer Panel, a ΔCq value below 2 is indicative of good DNA amplificability and predicts assay success with the TruSeq Amplicon kit. We compared DNA extracted with the Covaris truXTRAC® FFPE DNA Kit to DNA extracted with the QIAamp® DNA FFPE Tissue Kit from paired FFPE tissue samples.

Covaris truXTRAC is a highly reproducible method for the extraction and subsequent purification of DNA from FFPE tissue utilizing the Covaris Adaptive Focused Acoustics® (AFA®) technology. With AFA, a finely controlled focused acoustic energy field enables the removal and emulsification of the paraffin from the FFPE tissues. This efficiently deparaffinized tissue enables tissue rehydration, tissue digestion, crosslink reversal, and nucleic acid release.

Materials and Methods

DNA Purification

1. FFPE tissue blocks were stored at 4 °C upon delivery from Biotechnologies, Beltsville, MD, USA. Prior to sectioning, excess paraffin was trimmed from tissue blocks.
2. For each tissue type, a microtome was used to section 4 adjacent cuts of 15 μm.

3. For each tissue type, FFPE DNA was isolated with Covaris truXTRAC FFPE DNA Kit for sections #1 and #3 and with QIAamp DNA FFPE Tissue Kit for sections #2 and #4. The manufacturer's instructions for each Kit were followed.
4. The concentration of extracted DNA was determined using the Qubit Quant-it dsDNA BR assay.

Illumina FFPE QC Kit Setup

See manufacturer instruction for complete details (2)
(PN WG-321-1001)

1. All DNA samples were normalized to 1 ng/μl
2. For each sample, the qPCR mix was set up in triplicates as follows: H_2O - 5 μl, 2x buffer - 12.5 μl, 4x Primer - 2.5 μl, and DNA - 5 μl.
3. qPCR was run with the following profile and repeated for 40 cycles: 95 °C for 30s, 57 °C for 30s, and 72 °C for 30s.

Data Analysis

In the Illumina FFPE QC kit data analysis, Ct (threshold cycle) is referred to as Cq (quantification cycle)

1. Cq values were determined for each well.
2. Average Cq values were calculated for each tissue type and extraction method, as well as for the kit DNA standard.
3. Average Cq value for the DNA Standard was subtracted from the average Cq value for each FFPE sample to calculate the ΔCq
4. All samples with ΔCq value below or equal to 2 are considered of sufficient quality for use in TruSeq Amplicon library preparation.
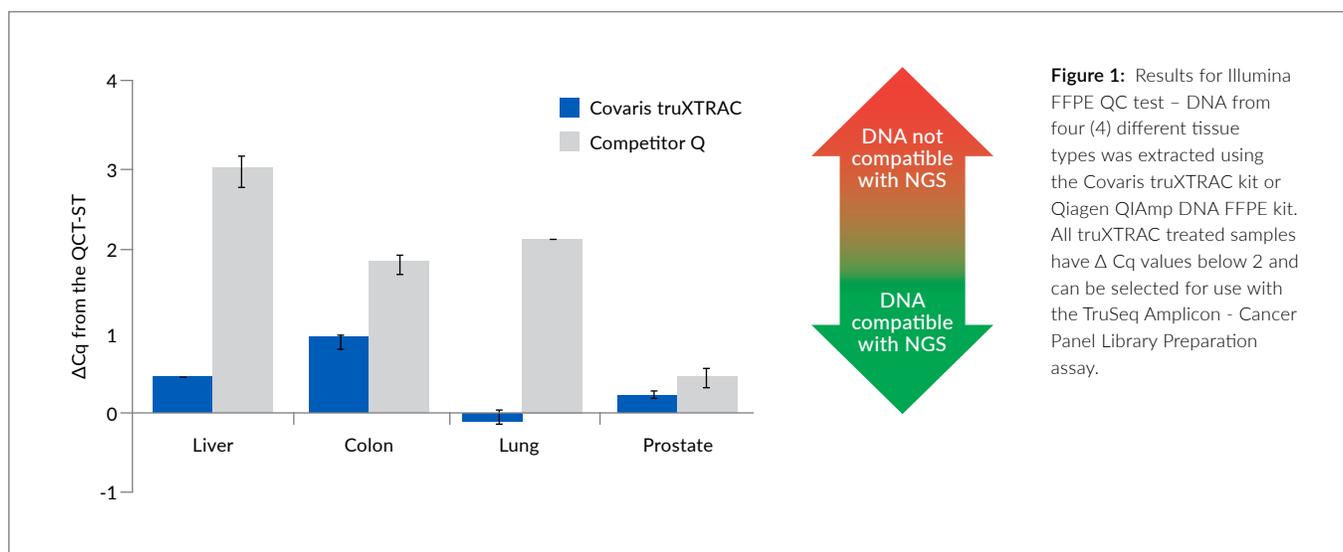
Figure 1: Results for Illumina FFPE QC test – DNA from four (4) different tissue types was extracted using the Covaris truXTRAC kit or Qiagen QIAmp DNA FFPE kit. All truXTRAC treated samples have Δ Cq values below 2 and can be selected for use with the TruSeq Amplicon - Cancer Panel Library Preparation assay.

Results

For all tissue types analyzed, DNA extracted with the Covaris truXTRAC Kit has a ΔCq below 1. However, DNA extracted with the QIAGEN QIAamp Kit has a ΔCq above 2 for Liver and Lung tissue, of 1.85 for Colon, and below 1 for Prostate.

All samples extracted with Covaris truXTRAC have a ΔCq value below 2 and so can be selected for library construction with TruSeq Amplicon - Cancer Panel, whereas only 50% of the samples extracted with the QIAGEN technique pass the quality threshold required for library construction.

Indeed, for other NGS library construction methods, a more stringent ΔCq cut-off may be appropriate for the specific requirements of the application in which case the low ΔCq possible with Covaris truXTRAC becomes more significant.

Conclusion

These results show that DNA extracted using the truXTRAC FFPE DNA kit is of higher quality than DNA extracted using the Qiagen FFPE kit. Critical NGS applications require a highly amplifiable DNA template that traditional methods, with harsh paraffin removal conditions, and incomplete tissue rehydration cannot achieve. DNA extraction with truXTRAC gently removes the paraffin, without heat or organic solvent, and simultaneously rehydrates the tissue, thus making crosslink reversal more complete. Overall, it results in high quality DNA which in turn can yield better Illumina NGS libraries.

It is of critical importance to develop relevant metrics for sample preparation for NGS analytics. For example, DNA yield from FFPE tissue was a historically a primary metric, however, is has been shown to be irrelevant for FFPE DNA when carried out with a UV based measurements (1). But even if the quantification is carried out with a fluorometric assay, this is not relevant if the extracted DNA is not usable in sequencing analysis, thus the importance to develop new QC metrics to define NGS-grade sample preparation.

References

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2. Using the Illumina FFPE QC kit with the truSeq Amplicon-Caner panel. http://support.illumina.com/content/dam/illumina-support/documents/myillumina/255af045-4832-44e6-af20-ee4f28956b5e/truseq_amplicon_cancer_panel_ffpe_qc_insert_15033035_a.pdf