High Quality DNA and RNA Extraction and Purification from FFPE Samples with Covaris truXTRAC® FFPE tNA Ultra Kit – Magnetic Beads

Abstract: The following application note introduces a semi-automated workflow using the Covaris ML230 Focused-ultrasonicator with the novel truXTRAC tNA Ultra Kit featuring the AFA-TUBE PP Screw-Cap 0.5 ml paired with ThermoFisher's KingFisher Duo to co-extract DNA and RNA from the same FFPE sample. This workflow allows flexibility in sample batching, faster parallel processing, and high-quality nucleic acid extraction, ideal for next generation sequencing library construction. The newly designed AFA-TUBE PP Screw-Cap 0.5 ml allows easy FFPE sample collection, does not require a transfer step before processing on a Covaris Focused-ultrasonicator, and can be integrated into a fully automated workflow on a liquid handler.

Introduction

As the most prevalent method of preserving and archiving solid tissues, Formalin Fixed Paraffin Embedded (FFPE) tissue samples have become the primary sample type for clinical oncology NGS based assays [1]. As the demand for clinical oncology sequencing has increased dramatically, clinical sequencing labs receive FFPE sections without having significant control over how the samples are shipped and stored [2]. This can impact sample processing efficiency, sample loss probability during transfer, and automated and semiautomated purification workflows. The optimized method we describe in this application note aims to:

1. Simplify the FFPE section sample collection and shipping by utilizing a Covaris screw cap AFA-TUBE PP Screw-Cap 0.5 ml. The AFA-TUBE PP Screw-Cap 0.5 ml features a large opening for sample collection and a conical shape which simplifies pipetting and compatibility with off the shelf labware.
2. The barcoded AFA-TUBE PP Screw-Cap 0.5 ml enables traceability of the sample for a more streamlined workflow at the clinical lab and minimizes human error.
3. The design of the AFA-TUBE PP Screw-Cap 0.5 ml allows easier automation integration.
4. Parallel processing up to six tubes in a strip format using the Covaris ML230 reduces hands on time and provides a faster workflow.

This application note demonstrates robust FFPE extraction yielding high quality nucleic acids. RNA passed DV_{200} (>20%) and FRQ (>0.2) quality metric thresholds recommended by Illumina and ThermoFisher, respectively [3]. DNA quantified by the KAPA hgDNA Quant and QC kit exhibited high amounts of amplifiable material (Q 129/41 ratio >0.4).

Materials

- ML230 Focused-ultrasonicator (PN 500656)
- truXTRAC FFPE tNA Ultra Kit – Magnetic Beads (PN 520304)
- ML230 Rack 6 AFA-TUBE PP Screw-Cap 0.5 ml (PN 500696)
- Qubit 4.0 Fluorometer (Thermofisher, Cat. Q33226)
- Roche LightCycler® 96 Real-Time PCR Cycler (Roche, Cat. 05815916001)
- Agilent 2100 Bioanalyzer Instrument (Agilent, Cat. G2939BA)
- TaqMan™ Fast Virus 1-Step Master Mix (Thermofisher, Cat. 4444432)
- 20X TaqMan Gene Expression Assay, GUSB (Thermofisher, Cat. 4331182)
- Promyelocytic Leukemia (HL-60) Total RNA (Thermofisher, Cat.AM7836)
- Qubit™ RNA BR Assay Kit (Thermofisher, Cat.Q10210)
- Qubit™ RNA HS Assay Kit (Thermofisher, Cat. Q32852)
- Agilent RNA 6000 Nano Kit (Agilent, Cat. 5067-1513)
- Qubit dsDNA HS Assay Kit (Thermofisher, Cat. Q32851)
- Qubit dsDNA BR Assay Kit (Thermifisher, Cat. Q32850)
- Agilent DNA 12000 Kit (Agilent, Cat. 5067-1509)
- KAPA Human Genomic DNA Quant & QC Kit (LightCycler® 480) (Roche, Cat. 07960620001)
- Eppendorf Adapter for 0.5 ml microcentrifuge tubes and 0.6 ml Microtainers® (Eppendorf, Cat. 22636227)

Methods

Tissue Handling: FFPE Blocks were stored at 4 °C upon arrival from CHTN (Cooperative Human Tissue Network, Eastern Division University of Pennsylvania). Before sectioning, excess paraffin was trimmed from the tissue blocks. One FFPE Block was used per tissue type. All scrolls were cut by utilizing a microtome on the same day and were randomized and stored in the AFA-TUBE PP Screw-Cap 0.5 ml at 4 °C prior to extraction (≤ 3 days). All extractions were performed using 1 x 10 µm scrolls per sample tube. The area of both, liver and kidney tissue, was 12 mm x 15 mm.

Experimental Details: The experimental workflow is shown in ***Figure 1***. Twelve FFPE tissue scrolls were treated in two batches of six samples on the Covaris ML230 Focused-ultrasonicator using the truXTRAC FFPE tNA Ultra Kit (PN 520304). Post AFA-enhanced paraffin emulsification, the samples underwent Proteinase K treatment followed by centrifugation. The workflow allows for parallel processing of centrifuged samples, wherein the supernatant was subjected to RNA purification steps and the tissue pellet was subjected to DNA purification steps.

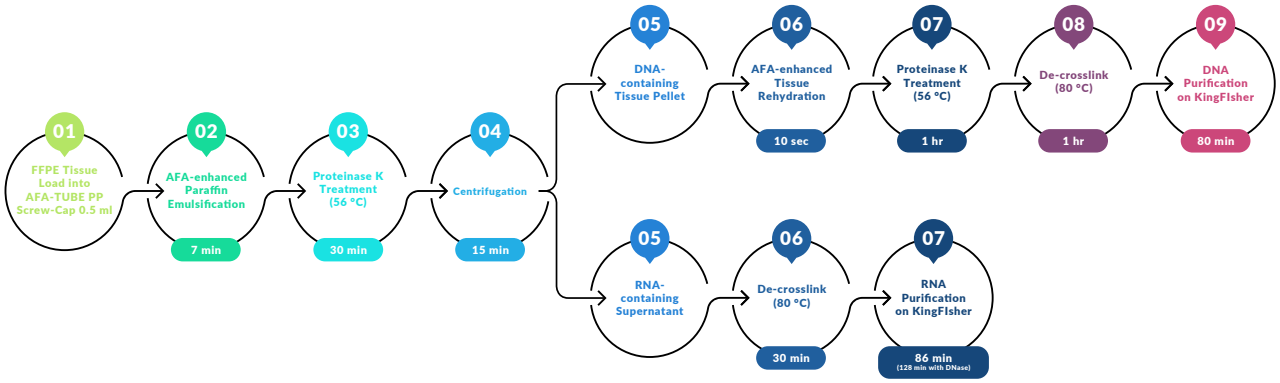

Figure 1. truXTRAC FFPE tNA Ultra Kit Workflow for a batch of 6 samples. Quick TAT of 7 minutes for upstream paraffin emulsification step allows for maximizing the capacity for downstream purification steps.

The turn-around-time (TAT) for AFA-enhanced paraffin emulsification for two batches of six samples on the Covaris ML230 Focused-ultrasonicator was 14 minutes. The total processing time to extract and purify the RNA samples was ~ 3.2 hours (without DNase treatment) and ~ 3.9 hours (with DNase treatment). The total processing time for extraction and purification of the DNA samples was ~ 5.1 hours. Whenever the DNA and RNA extraction can be parallelized, i.e. RNA purification on the KingFisher Duo during Proteinase K Treatment of the DNA, the total TAT would be ~ 5 hours. The actual hands-on time for this entire workflow was ~1.5 hours.

FFPE Tissue Extraction & Storage

DNA and RNA were extracted from two different FFPE tissue types and processed in 6 biological replicates. Each biological replicate was processed in triplicate when performing the FRQ assay, duplicate for KAPA Human Genomic DNA Quant and QC assay, and each biological replicated was processed once for all other metrics (N=6 per tissue). Using the truXTRAC FFPE tNA Plus Kit – Mag Beads with the KingFisher Duo, extractions were performed following the AFA settings describes in ***Table 1***.

Consumable	AFA-TUBE PP Screw-Cap 0.5 ml
Number of Samples per Treatment	6
PIP (W)	350
Duty Factor (%)	30
Cycle per Burst	1000
Duration (s)	420
Temperature (°C)	20
Lysis Buffer Volume (µL)	500

Table 1. The AFA Parameters used to emulsify the FFPE Tissue on the ML230.

DNase treatment was performed during the extraction of all RNA samples to eliminate DNA contamination. RNA samples were stored at -80 °C when not in use and on ice when in use. DNA samples were stored at -20 °C when not in use and on ice when in use.

Results

RNA Yield

RNA yield from the purified RNA samples was carried out using a Qubit BR (Broad Range) assay kit [4] since the RNA yield range from FFPE sections can vary widely depending on the quality of the FFPE block. Both tissue types yielded between 3 to 6 µg of RNA per 10 µm section (***Figure 2***).

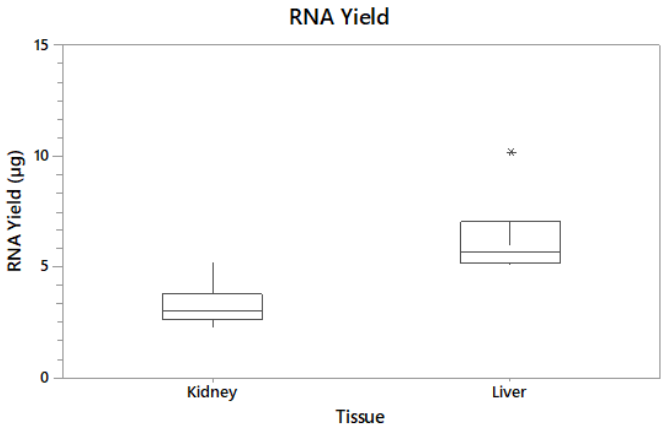

Figure 2. Boxplot of the RNA yield (µg) measured with the Qubit BR assay carried out on kidney and liver FFPE tissue samples (N = 6 per tissue type).

DNA Yield

DNA yield from the purified DNA samples was carried out using a Qubit BR (Broad Range) assay kit [5] since the range from FFPE sections can vary widely depending on the quality of the FFPE block. Both tissue types yielded ~1 µg of DNA per 10 µm section (***Figure 3***).

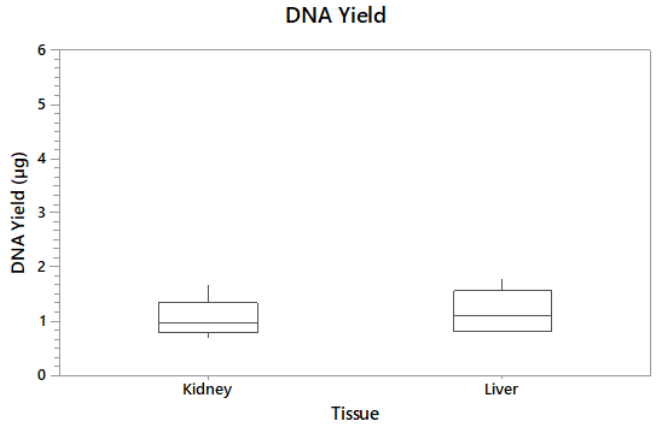

Figure 3. Boxplot of the DNA yield (µg) measured with the Qubit dsDNA BR Assay kit analysis from two tissue types (N = 6 per tissue type).

RNA Distribution Value 200 (DV_{200})

Samples were processed on the 2100 Bioanalyzer using the RNA 6000 Nano Kit to visualize RNA profiles [6]. Smear Analysis was performed on the Bioanalyzer software (***Figure 4***). RNA extracted from the Covaris truXTRAC FFPE tNA Plus Kit – Mag Beads Kit with the ML230 obtained a DV_{200} > 50% for all tissues and replicates tested, which is far above the Illumina recommendation (20%).

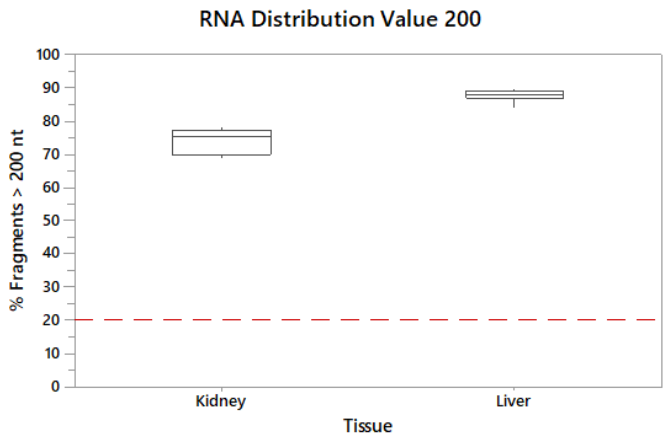

Figure 4. Boxplot of the DV_{200} scores determined by the Smear Analysis tool on the Agilent 2100 Bioanalyzer (200 to 8500 nt) for both tissue types (N = 6 per tissue type). All samples generate DV_{200} values above the red dashed line (minimum acceptable score threshold of 20%).

Functional RNA Quantitation (FRQ)

The FRQ RT-qPCR based assay was performed following the Ion Torrent (Thermo Fisher) Application Note [7]. A Roche LightCycler was used for the RT-qPCR analysis. Calculated FRQ scores were based on a calibration curve ranging from 0.02 ng/µL to 50 ng/µL. All extracted samples displayed more than double the quality acceptance criteria of 0.2 ng/µL, signifying high confidence that both tissue types will produce quality sequencing results (***Figure 5***).

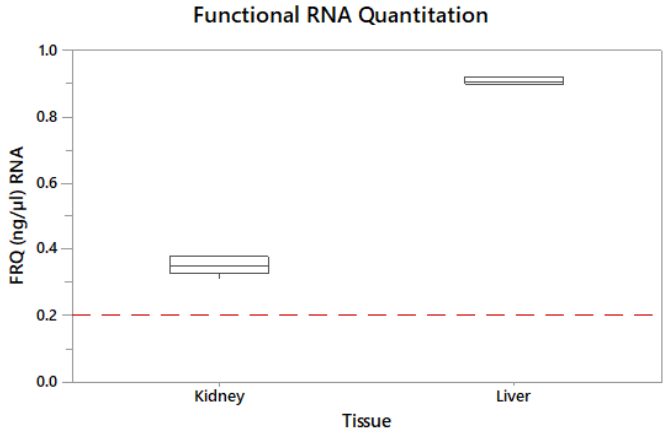

Figure 5. Boxplot of the Functional RNA Quantitation (FRQ) score for liver and kidney FFPE tissues. All samples generate values above the red dashed line (FRQ score minimum threshold of 0.2 ng/µl).

DNA Q-Ratio (129 bp/41 bp)

The quality of the genomic DNA was determined using the KAPA hgDNA Quant and QC kit [8]. The kit contains several qPCR primer pairs of varying amplicon sizes to determine the availability and amplifiability of different fragment ranges in the extracted FFPE DNA. Since poor DNA quality has a greater impact on the amplification of longer targets, the relative quality of an FFPE DNA sample can be inferred by normalizing the concentration obtained using the 129 bp assay against the concentration obtained from the 41 bp assay. This normalization generates a Q-ratio (with a value between 0 and 1) that is indicative of DNA quality, which correlate with the amount of amplifiable DNA.

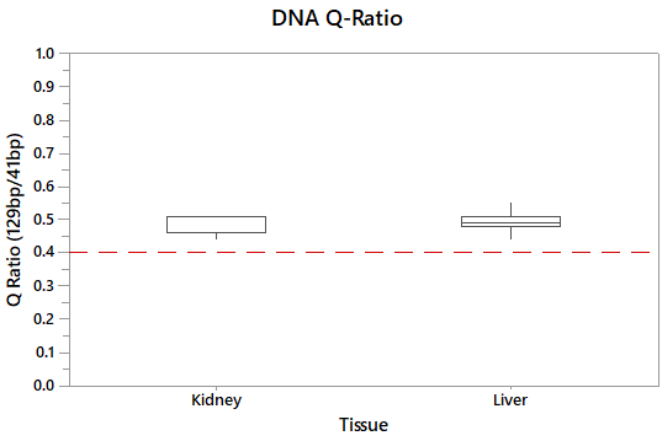

Figure 6. Boxplot of the KAPA hgDNA Quant and QC Q-ratio (129/41) analysis of the FFPE DNA extracted from kidney and liver FFPE tissue (N = 6 per tissue type). All samples generate values above the red dashed line (Q-ratio score minimum threshold of 0.4 ng/µl).

Conclusion

The newly developed Covaris truXTRAC tNA Ultra FFPE extraction method utilizing Covaris AFA-TUBE PP Screw-Cap 0.5 ml represents a complete workflow solution for easier sample collection, transportation, and active AFA-based paraffin emulsification in a single tube. In addition, it allows the extraction of high-quality DNA and RNA for NGS compatible workflows. More importantly, the use of the Covaris AFA-TUBE PP Screw-Cap 0.5 ml enables semi-automated and automated FFPE RNA and DNA purification workflows with easy integration and faster processing.

References

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