Robust Sample Preparation Workflows for Every Analyte from FFPE Samples

Brian Russo | Covaris, LLC, Woburn, Massachusetts 01801

Robust Workflows for Extraction, Isolation, and Purification of Every Analyte from Complex FFPE Samples

Introduction

Formalin-fixation and paraffin-embedding (FFPE) of tissue samples are routinely used in clinical histopathology. While FFPE preserves tissue architecture, removal of paraffin is an essential prerequisite to achieve high quality LC-MS data. Xylol, despite all its efficacy presents severe health risks and repeated steps of xylol removal compromises assay reproducibility. Superior sample preparation not only ensures removal of unwanted matrix components, but also ensures reliability, reproducibility and robustness of the assays. Adaptive Focused Acoustics® (AFA®) Technology (***Figure 1***) works via a focused short-wavelength energy that can be efficiently used not only for lysis of samples, but also for active deparaffinization, and extraction, isolation, purification of critical analytes (DNA, RNA, Proteins) from any tissue samples. In this study, we highlight two different workflows that ensure - i) generation of high quality and yield of tNA (Total nucleic acids - DNA and RNA), and ii) extraction, purification, and digestion of global or targeted proteins - from any tissue types in FFPE.

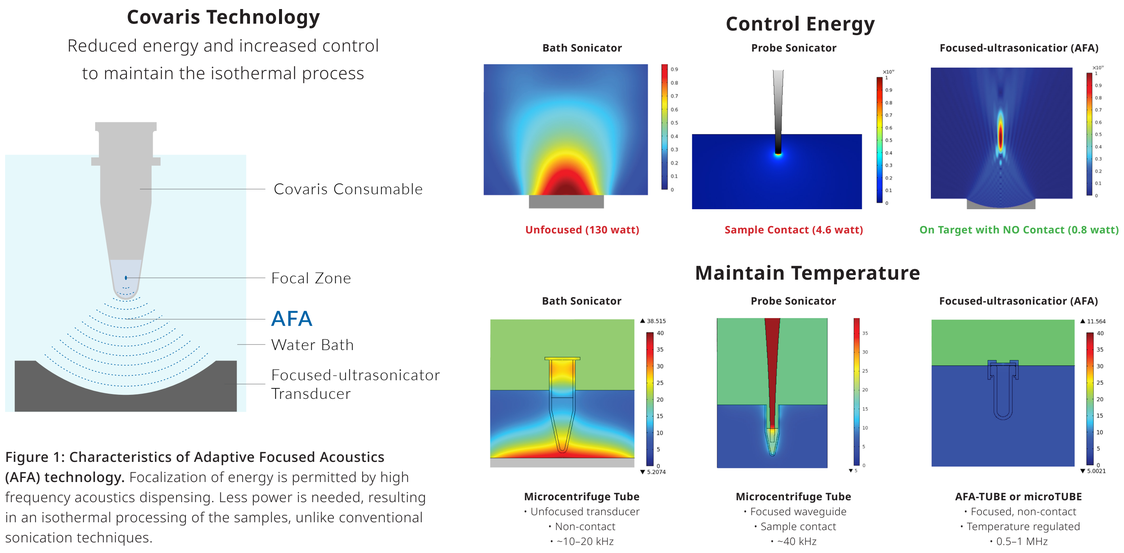

Figure 1: Characteristics of Adaptive Focused Acoustics (AFA) technology. Focalization of energy is permitted by high frequency acoustics dispensing. Less power is needed, resulting in an isothermal processing of the samples, unlike conventional sonication techniques.

Key Takeaways

Covaris promotes robust, easy, and scalable sample preparation through:

- Standardized tissue/cell disruption
  - Improved consistency and reproducibility
  - Hands-free, automated
  - Processing in different formats, from single tube up to 96-well plate, with same the sample-to-sample quality
- Fast, non-contact, easy, cold, and reproducible extraction for all soft and hard tissue samples
  - Use your buffer of choice
  - Scale up from single tube to 96-well format and beyond
  - Includes non-mammalian samples like plants, yeast, and bacteria
- Active, non-toxic deparaffinization and improved rehydration of FFPE and LCM tissue
  - Enables “fresh frozen like” protein extraction from FFPE samples, without any organic solvent

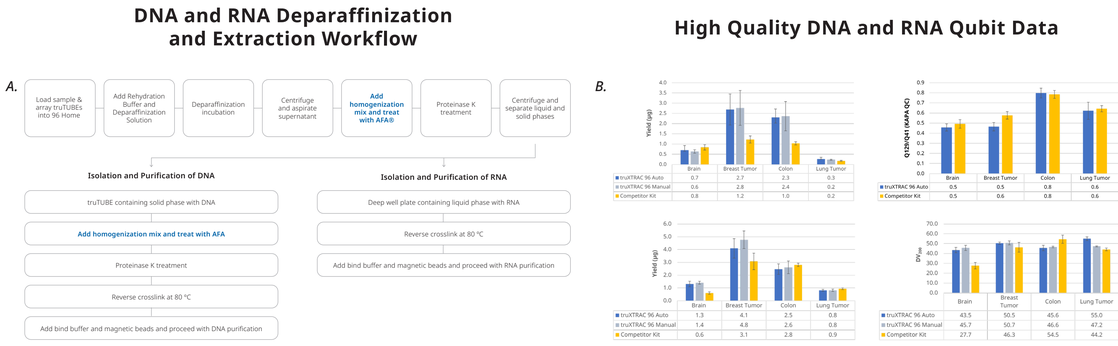

Figure 2. ***A.*** Deparaffinization and extraction workflow for DNA and RNA from the same tissue scroll. ***B.*** High quality and yield of DNA (yield: Qubit; quality: DNA KAPA QC (129/141 ratio @ 1 ng input) and RNA yield: Qubit; quality: RNA DV_{200} scores with Bioanalyzer) with Automated or Manual workflows.

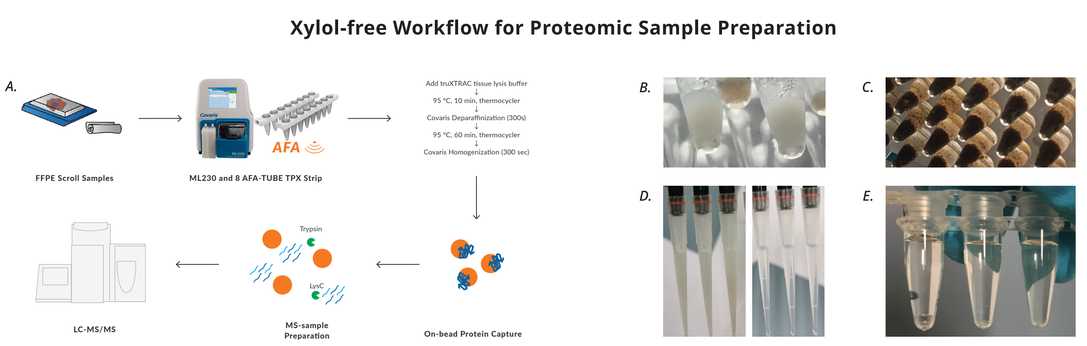

Figure 3. ***A.*** Workflow of the xylol-free APAC method for efficient proteomic sample preparation of FFPE tissue in 96-well format. Overview of the sample preparation workflow; ***B.*** A combination of heating and AFA-sonication removes paraffin from FFPE tissue. ***C.*** Protein precipitation on magnetic beads using the Covaris 96-well plates. ***D.*** Fast and parallel removal of paraffin. ***E.*** Final workflow step after tryptic digest showing a clear solution without residual paraffin.

Methods

tNA: For this study, all samples were prepared following the standardized workflow[1] (***Figure 2A***). The quality of DNA and RNA were monitored using KAPA QC and DV_{200} scores, respectively (***Figure 2B***). The yield of both DNA and RNA were monitored using Qubit (***Figure 2B***).
Proteins: Details of the workflow for extraction, purification, and digestion of proteins has been published elsewhere[2] (***Figure 3***). FFPE tissue scrolls were used in a Covaris Plate, incubated post-treatment with “Covaris Tissue Lysis Buffer”, processed with Covaris LE220-Plus Focused-ultrasonicator for deparaffinization and DNA shearing. All samples were measured on a 100 min. gradient using a Q Exactive™ HF-X Orbitrap™ High Resolution Accurate Mass Spectrometer coupled to an EASY-nLC™ 1200 UHPLC.

Discussion

A non-toxic and robust deparaffinization strategy for FFPE samples for either genomic sequencing (tNA) or LC-MS-based proteomics is showcased. This sample preparation method combines fast emulsification of paraffin with AFA followed by extraction, isolation, and purification of analytes (tNA or Proteins) in a highly consistent, reproducible and sensitive way.

The two sample preparation strategies described here addresses unprocessed FFPE tissue resulting in either clean nucleotides or clean peptides. While being highly reproducible, scalable and robust, our non-toxic deparaffinization and sample preparation workflow generates similar yields to common xylol-based protocols.

Covaris truXTRAC® FFPE SMART Solutions offer a scalable approach to deparaffinization, extraction, and purification of both DNA and RNA from FFPE samples in an automation-friendly, highly adaptable workflow. Covaris’ FFPE Protein workflows offer a scalable approach to deparaffinization followed by extraction, purification, and digestion of proteins in the same 96-well plate thereby minimizing sample loss, while ensuring high protein and peptide yield.

References

1. truXTRAC® FFPE SMART Solutions Comprehensive Solutions Active Paraffin Removal with AFA® Reproducible Results Time and Cost Savings High-Quality Analytes for Reliable Downstream Analysis. Accessed June 16, 2023. https://www.covaris.com/wp/wp-content/uploads/m020162.pdf
2. Schweizer L, Coscia F, Müller J, Doll S, Wierer M, Mann M. Accessed June 16, 2023. https://www.covaris.com/wp/wp-content/uploads/2020/06/M020141.pdf