Novel, Automated Co-extraction of High-quality DNA and RNA from a Single FFPE Sample

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Abstract

Formalin-fixed, paraffin-embedded (FFPE) tissue preservation is the preferred method to archive clinical tissue biopsy samples for histopathological diagnosis. As advances in clinical molecular pathology continue to grow, the importance of reliable methods of extraction from FFPE tissue specimens becomes vital to ensure that patients receive timely and accurate reports. However, nuclear acid extraction from FFPE samples can be challenging and labor-intensive, often resulting in degraded and fragmented DNA and RNA. Given the precious and limited availability of these clinical samples, the ability to differentially co-extract high-yield and high quality DNA and RNA from a single sample input provides a tremendous advantage.Coupling the Covaris LE220R-plus Focused-ultrasonicator with liquid handling automation and the truXTRAC® FFPE kits for high-yield co-extraction, in this poster we demonstrate a standardized clinical FFPE extraction workflow providing downstream result confidence (higher yields and corresponding higher DV2000 scores), increased efficiency, decreased sample variability, and manual touchpoints throughout the process. Furthermore, it shows that the automated DNA and RNA workflows yield similar results as compared to manual methods using our truXTRAC FFPE kits.

Introduction: Covaris Adaptive Focused Acoustics® (AFA®) Technology

- Advanced acoustic technology enabling non-contact mechanical processing of samples
- Controlled bursts of focused high-frequency acoustic energy for efficient, reproducible, and isothermal sample processing (Figure 1)
- Enables the acoustic energy to be focused into a discrete focal zone within a sample vessel
- Minimal energy input, avoiding the adverse effects heat and sample over-processing typical of ordinary sonicators

Covaris truXTRAC FFPE total Nucleic Acid Kit

- Designed for efficient and simultaneous extraction of total nuclear acids (DNA and RNA) from FFPE tissue samples using Covaris AFA technology platform (Figure 2)
- Enables the active, organic solvent-free removal of paraffin from FFPE tissue samples in an aqueous buffer
- Active tissue rehydration
- Reversal of formaldehyde crosslinks to improve extraction/purification of longer transcripts (increased DV2000 scores)

RNA and DNA Yield & Quality

As it has been well documented, FFPE sample storage and difficulty in processing can lead to nucleic acid degradation, often resulting in fragmented RNA transcripts. The assessment of RNA quality has traditionally been performed using the RNA Integrity Number (RIN) from Agilent, but recent studies have shown that mean RNA fragment size is a better and more reliable quality determinant for RNA quality. The DV2000 metric (Cheiling, OmiciaCo, Evaluation, Intersil Report, Bostian, Guarde, Ireland, and Agilent are registered trademarks of their respective owners).

truXTRAC FFPE total NA Kit Manual vs. Automated Clinical Workflow Comparison

Through a collaboration with OmniSeq®, the LE220R-plus Focused-ultrasonicator was integrated with liquid handling automation for scalability and throughput, sample tracking and workflow robustness, and reproducibility. As an added benefit, OmniSeq did not observe lower DNA and RNA extraction yields when comparing the manual and automated workflow. As a result, similar CDNA and DNA library preparation yields were obtained using the truXTRAC FFPE total NA kit. Furthermore, comparable downstream sequencing results were obtained (Table 1 through 3).

Conclusion

In this poster, we have demonstrated the use of Covaris AFA technology for de-paraffinization and tissue rehydration in combination with the Covaris truXTRAC FFPE kits for DNA and RNA co-extraction from FFPE tissue samples. Both the manual and automated methods are amenable to clinical workflows where the most challenging FFPE sample types are being examined. The truXTRAC FFPE total NA kit provides co-extraction of high-quality DNA and RNA yields similar or better than alternative methods. More specifically for RNA extraction, the DV2000 scores for Covaris truXTRAC FFPE processed samples are higher for the majority of the FFPE sample types studied and above the recommended threshold level for high-quality downstream sequencing, as compared to Competitor Q. The integration of the Covaris LE220R-plus instrument with liquid handling automation in concert with a unique co-extraction process based on the truXTRAC FFPE kits enables the standardization of a clinical FFPE extraction workflow that provides downstream result confidence (higher yields) and corresponding higher DV2000 scores, increased efficiency, and decreased sample variability; thus allowing better clinical analysis and more robust outcomes.

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