Total Nucleic Acids Extraction from FFPE Samples using Adaptive Focused Acoustics® (AFA®) Technology for Comprehensive Genomic Profiling

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Comprehensive Genomics Profiling (CGP) is the standard of care for cancer that allows molecular pathology laboratories consolidate individual biomarkers into a Next-Generation Sequencing (NGS) assay (Dy, Nesline et al. 2019, Nesline, DePietro et al. 2019). Standard formalin-fixed paraffin-embedded (FFPE) methods are used for preserving human tissue specimens and helps mitigate risks of infectious contaminating agents and preservation of cellular architectural components. However, FFPE offers several challenges – inefficient removal of paraffin; retrieving adequate amounts and quality of DNA and RNA; reducing workflow complexity, turnaround time, and increasing throughput.

Covaris' truXTRAC® FFPE SMART Solution offers superior performance in ensuring high-yield and quality for nucleic acids, while addressing the shortfalls of other commercial FFPE extraction kits: truXTRAC FFPE SMART Solution offers a flexible, automatable workflow - a robust all-inclusive workflow from sample deparaffinization to purified DNA and RNA.

A fully automated FFPE sample extraction solution has been adopted for the Omniseq INSIGHT® clinically approved CGP test (Labcorp), which incorporates the TruSight® Oncology (TSO) 500 assay (Illumina). Omniseq INSIGHT utilizes both RNA and DNA to interrogate the full exonic coding region of 523 genes by NGS for mutations, select copy number alterations, fusions/splice variants, microsatellite instability tumor mutational burden, expression of 64 immune genes, and PD-L1 by immunohistochemistry. In addition, Omniseq INSIGHT allows for processing of up to 70 clinical samples in a single NovaSeq® 6000TM sequencing run with a turnaround time of <7 days from specimen receipt to report, with as low as 40ng DNA and 20ng RNA inputs (Conroy JM et al, 2021).

This study reports performance of the novel truXTRAC FFPE SMART Solution from FFPE tissue slide scrapings with analysis using the TSO 500 pipeline. The FFPE samples were divided into two groups – (GEN1) samples prepared using Covaris filter-based chemistry and the Lynx® automated liquid handler (Dynamic Devices) using the same pipeline as described by Conroy JM et al, (Conroy, Pabla et al. 2021); (GEN3) samples prepared using truXTRAC FFPE SMART workflow. DNA and RNA quantity, as measured by picogreen and ribogreen staining, demonstrated higher yields with the GEN3 chemistry.

For QC analysis of 10 DNA samples using TSO 500, GEN3 samples outperformed GEN1 samples across all key QC parameters demonstrating a tighter distribution of coverage, insert size, and useable MSI sites, without affecting data quality. QC analysis across RNA samples using TSO 500 revealed robust fusion detection correlation and improved QC metrics between the GEN3 and GEN1 chemistries. The truXTRAC FFPE SMART Solution offers a robust, reliable, reproducible, and automation-compatible workflow enabling extraction and purification of DNA and RNA resulting in overall improved TSO 500 assay performance.