Fully Automated Extraction of High-Quality Total Nucleic Acids from FFPE Specimens using Covaris truXTRAC® FFPE SMART Solutions & Hamilton Robotics

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Overview

- Covaris, Hamilton, and Labcorp (OmniSeq®) solution merges Hamilton's Microlab® STAR™ with Covaris' truXTRAC® FFPE SMART Solutions technology for FFPE processing
- Highlights the potential of the Assay-Ready Workstation (ARW) in improving FFPE DNA/RNA extraction processes, including those in the OmniSeq INSIGHT assay workflow
- DNA and RNA from FFPE Specimens purified automatically by ARW
- DNA and RNA quality metrics and yield analyzed at Labcorp
- TruSight® Oncology 500 (TSO 500, Illumina®) next-generation sequencing (NGS) metrics analyzed by Labcorp
- Samples extracted using the ARW and truXTRAC FFPE SMART Solutions outperform the current clinically-validated extraction workflow in terms of yield, purity, and downstream DNA and RNA NGS performance

Introduction

- Comprehensive Genomic Profiling (CGP) of solid tumors with NGS is crucial for assessing multiple biomarkers in cancer patients' tumors (1,2)
- Extracting nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissues presents challenges due to high specimen heterogeneity, leading to compromised quality and limited material for NGS testing
- The automated ARW system, integrated into the OmniSeq CGP workflow, addresses these challenges by efficiently co-extracting DNA and RNA from FFPE tissues (3-5)

Performance

- Higher yield from small samples
- Superior DNA/RNA quality
- Enables biomarker discovery

Highest yield and quality of analytes for sequencing; Lower failure rates

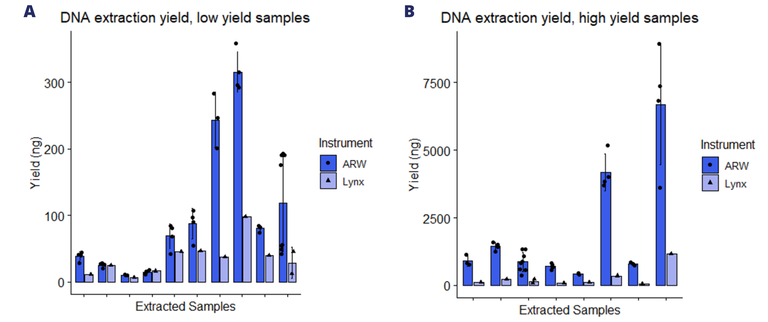

Figure 1: DNA yields analyzed from 120 clinical samples subjected to concurrent DNA and RNA co-extraction using the established automated clinical workflow (Dynamic Devices® Lynx®) and the newly developed ARW. ***A***. DNA yields observed from low input and low yield samples. ***B.*** DNA yields observed from high yield and high input samples. These findings indicate that the ARW DNA extraction successfully meets Omniseq clinical standards.

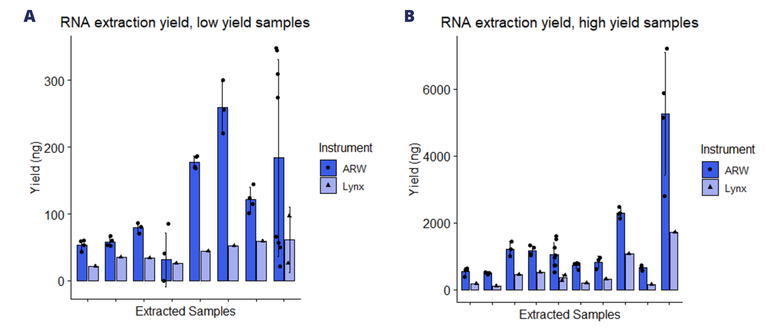

Figure 2: RNA yields assessed from 120 clinical samples with co-extracted DNA and RNA using established automated clinical workflow (Lynx) and the new ARW method. ***A***. RNA yields from low input and low yield samples. ***B***. RNA yields from high yield and high input samples. The results demonstrate that ARW DNA extraction yields meet OmniSeq clinical standards.

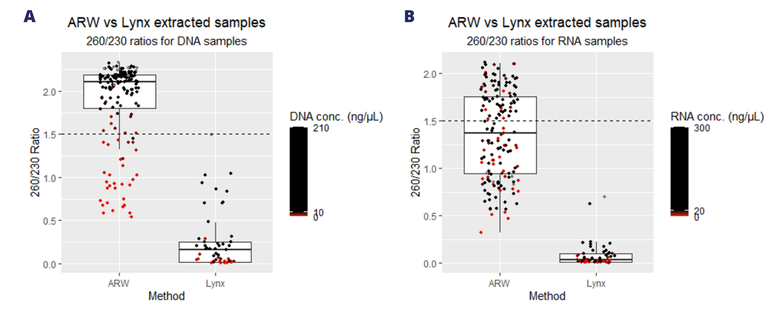

Figure 3: NanoDrop™ measurements depict the 260/230 Ratios for DNA (***A***) and RNA (***B***). Individual value points are color-coded based on concentration according to the colorbar located to the right of each graph. These data confirm the ARW extraction purity meets OmniSeq INSIGHT clinical standards.

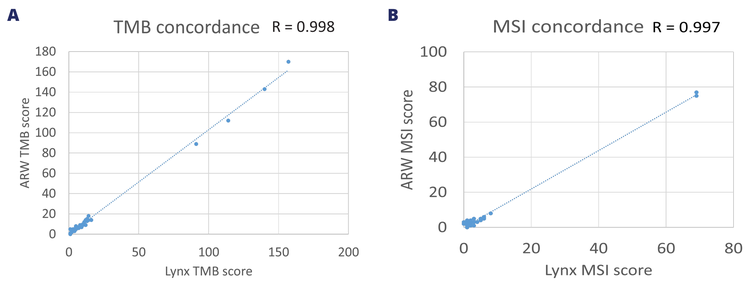

Figure 4: NGS concordance data for Tumor Mutation Burden (TMB) and microsatellite instability (MSI) for 86 Clinical samples sequenced with TSO 500 assay. ***A***. TMB concordance between the established automated clinical workflow (Lynx) and ARW shows high correlation between the two methods. ***B***. MSI concordance indicates comparable performance between Lynx and ARW methods.

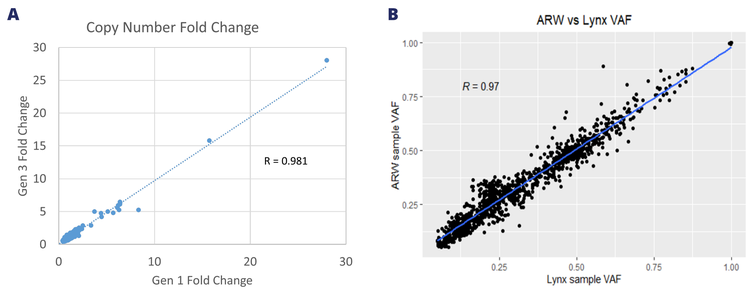

Figure 5: Concordance data across 523 genes for Copy Number Variation and Small Variant calling from 86 clinically sequenced samples using TSO 500 illustrates comparable performance. ***A***. Copy Number Fold Change data exhibits consistency between methods. ***B***. Summary data displays high concordance (>97% PPA and 100% NPA) in detecting Insertions, Deletions, and Substitutions, affirming method reliability.

Workflow

- Non-toxic, easy deparaffinization
- Flexible automation

Streamlining of disjointed workflows; Manual and automated options

	Covaris Hamilton ARW
Batch Size	16 24 48 96
Throughput	
Integration time	
Covaris and Hamilton Support	★ ★ ★ ★ ★

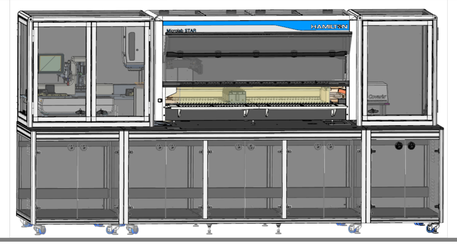

Figure 6: The ARW System, comprising the Hamilton Microlab® STAR™ with the integrated R230, LabElite ID Capper, and Covaris accessories, offers a seamlessly integrated solution. With collaborative support from Covaris and Hamilton, this system validated by Labcorp can be efficiently replicated with minimal time investment.

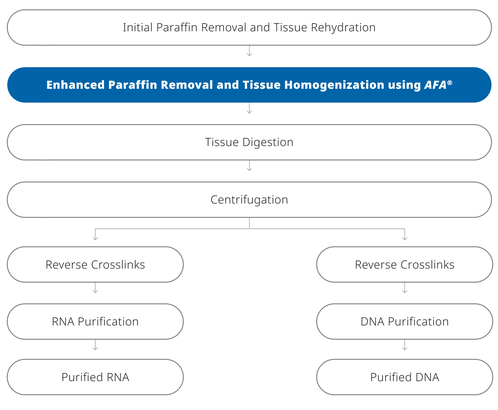

Figure 7: truXTRAC FFPE SMART Solutions Workflow: Efficient extraction of DNA and RNA. This diagram illustrates the process for obtaining high-quality and high-yield DNA and RNA simultaneously using Covaris truXTRAC FFPE SMART Solutions with Adaptive Focused Acoustics® (AFA®) technology. The ARW featured at Labcorp automates this workflow efficiently.

Materials and Methods

For the ARW extraction process, FFPE tumor slide specimens were carefully scraped into Covaris truTUBEs and then placed on the ARW deck following the layout provided by the software prompts. Utilizing the truXTRAC FFPE SMART Solutions workflow, the samples underwent automated purification on the ARW system. Post-purification, four manual transfer steps were executed to a plate centrifuge for further processing.

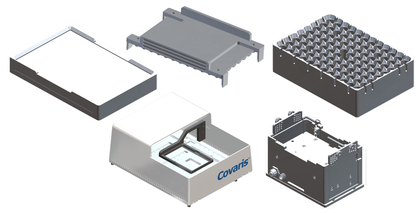

Figure 8: Covaris on-deck components (clockwise from top left): Sponge Station, Shuttle Weight, 96 Dual Heat Block, Automation Universal Downholder, R230.

In contrast, for the Lynx extraction, paired FFPE tumor slide specimens were scraped into Covaris microTUBE-130 Snap-cap tubes and processed using the Clinical Extraction Method following Labcorp's Omniseq INSIGHT workflow.

Various analyses were conducted according to vendor specifications: RNA Yield was quantified using the Qubit™ RNA High Sensitivity (HS) Assay Kit (ThermoFisher), while DNA Yield was assessed using the Qubit™ 1X dsDNA High Sensitivity Assay Kit (ThermoFisher). For RNA and DNA purity, NanoDrop (ThermoFisher) A260/A280 and A260/A230 measurements were determined.

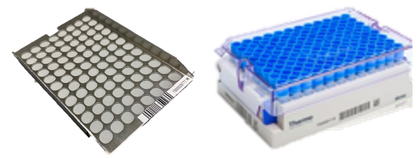

Figure 9: Covaris truXTRAC FFPE SMART Solutions Automation-Compatible Consumables

NGS was performed by Labcorp using the TSO 500 assay as per standard operating procedure. RNA and DNA TSO 500 QC Metrics were compared to those acquired from the established Lynx clinical workflow to analyze and evaluate the outcomes between the paired samples run on each extraction platform. Genomic alterations such as TMB, MSI, Copy Number, fusions and small variant detection were analyzed for concordance using Pearson correlation (R), positive percent agreement (PPA) and negative percent agreement (NPA).

Economics

- Reducing input requirement
- Reducing sequencing failures

Empowers budget-conscious customers by allowing scalable solutions

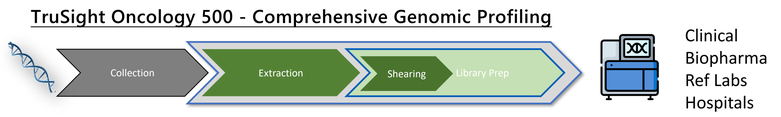

Figure 10: Overview of the TSO 500 Workflow: Illustrating the sample preparation steps prior to library sequencing.

Conclusions

- The ARW system offers full automation for concurrent extraction of high-quality DNA and RNA from FFPE tissue, seamlessly aligning with OmniSeq's established clinical CGP workflow (3-5).
- This system demonstrates substantial potential in enhancing FFPE nucleic acid extraction processes, especially within the OmniSeq INSIGHT framework.
- Samples extracted using the ARW, combined with truXTRAC FFPE SMART Solutions, outperform the established clinically validated extraction workflows for yield and in purity. They exhibit comparable efficacy in downstream DNA and RNA TSO 500 NGS analyses.
- This user-friendly, labor-saving, and high-throughput workflow ensures scalability and robustness. It facilitates reliable extraction even with low mass inputs like Fine Needle Aspirate FFPE samples, reducing the QNS rate and preventing resequencing costs and failures.
- The ARW consistently delivers robust nucleic acid yields, promising maximal biomarker detection and actionable insights for improved cancer patient outcomes.

References

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