High-fidelity Multiomic Profiling from FFPE Samples Using a Complete Co-extraction and Library Preparation Workflow

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Introduction

Solving FFPE Challenges in Comprehensive Genomic Profiling (CGP)

- FFPE fixation introduces degradation and chemical damage that compromise DNA and RNA integrity, leading to uneven coverage, variable insert sizes, and reduced sensitivity for variant and fusion detection.
- Conventional FFPE workflows are typically single-analyte and rely on chemically fragmented nucleic acids, increasing variability, sample consumption, and performance loss in challenging genomic regions.
- We present an integrated, automated DNA-RNA co-extraction and library preparation workflow enabling high-fidelity multiomic profiling from FFPE tissue with improved coverage uniformity, variant calling accuracy, and fusion detection sensitivity.

Materials and Methods

- **Samples:** Externally sourced FFPE scrolls (10–15 µm thick), n=48 for DNA extraction and WGS, n=28 for RNA-Seq
- **FFPE extraction:** Automated truXTRAC® FFPE SMART Total NA (Hamilton® Sonication STAR™) vs Supplier A DNA/RNA FFPE kit
- **WGS library preparation:** truCOVER® WGS Library Prep Kit with Amplification vs Supplier B Kit (100 ng input, 8 amplification cycles)
- **RNA library preparation:** truCOVER Total RNA Kit vs Supplier C and Supplier D kits (50 ng input)
- **Analysis:** Illumina® NovaSeq™ X Plus (30× coverage, WGS); NextSeq™ 2000 (20–25 million reads/sample, RNA-Seq); analysis via CLC Genomics Workbench and hap.py (DNA) and nf-core pipeline (RNA)

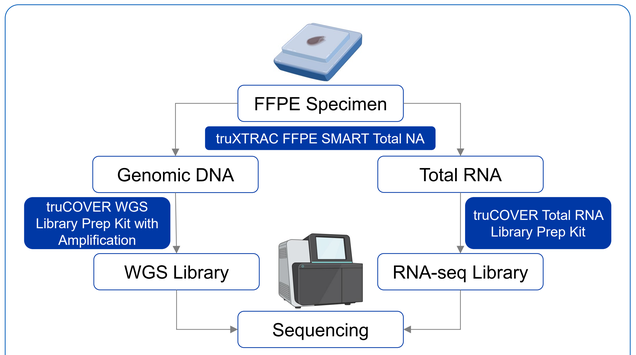

Figure 1. Proposed workflow for multiomic data generation from a single FFPE specimen. Icons adapted from Biorender.com.

Results

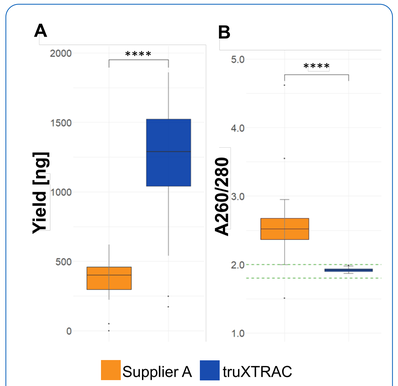

Figure 2. DNA extraction: (**A**) yield and (**B**) purity (A260/280) values.

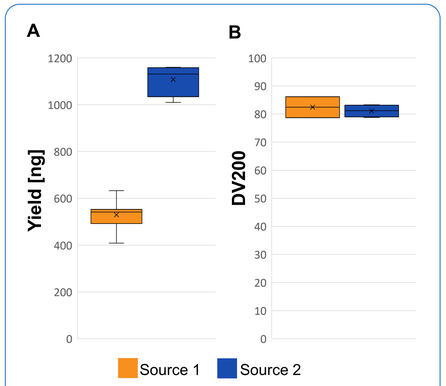

Figure 3. RNA extraction: (**A**) yield and (**B**) quality (DV200) values.

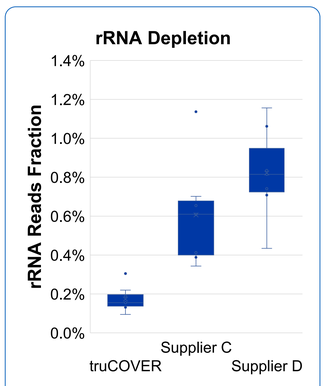

Figure 4. rRNA depletion efficiency

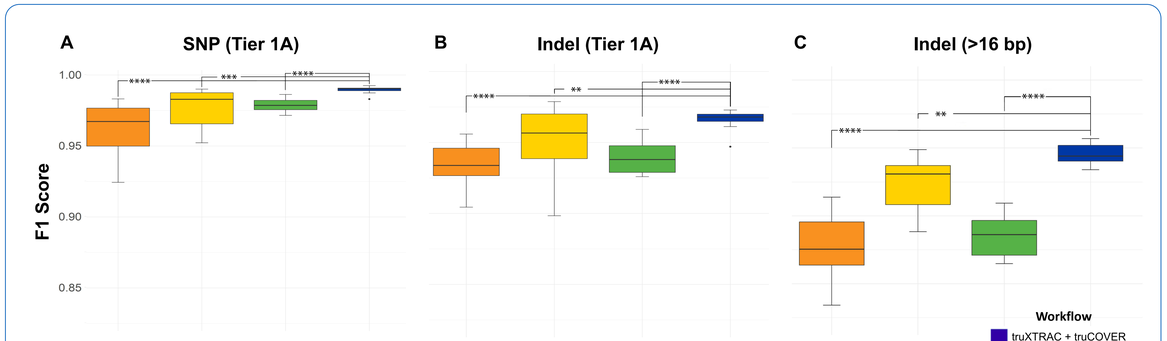

Figure 5. F1 score (harmonic mean of Precision and Recall) for WGS variant calling obtained from samples prepared in various Extraction + Library Prep workflows. (**A, B**) Scores for Tier 1A regions identified by OncoKB's list of druggable targets. (**C**) Scores for challenging indels, identified by extended length.

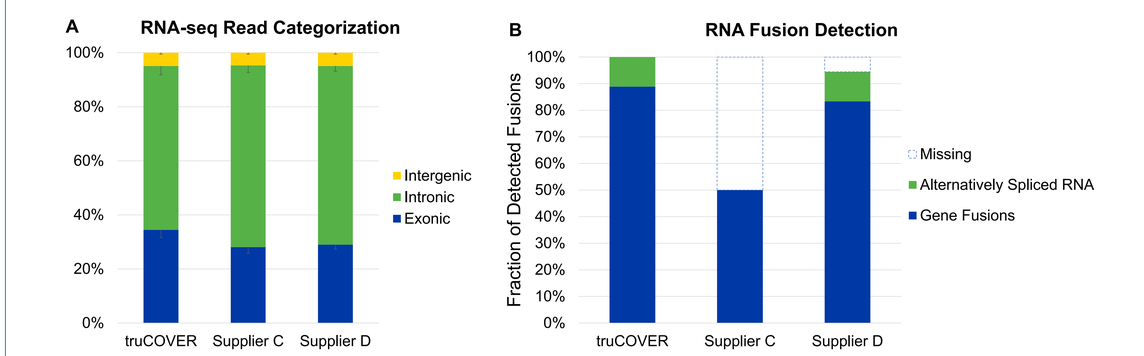

Figure 6. RNA-seq metrics used in clinical diagnostics. The truCOVER workflow results in **(A)** higher proportion of exonic reads compared with other tested methods and **(B)** libraries of ~350 bp, enabling detection of 100% of clinically relevant RNA fusions (reference material: SeraCare® Material No. 0710-0496)

Conclusions

- **Integrated FFPE multiomics:** Automated DNA and RNA co-extraction (Hamilton Sonication STAR) enables parallel analysis from a single specimen reducing tissue consumption and variability.
- **Superior DNA coverage performance:** Improved coverage uniformity supports reliable detection of SNPs and large INDELs (>16 bp) from degraded FFPE DNA.
- **Variant calling accuracy:** Statistically significant improvements in precision, recall, and F1 score are observed for clinically actionable variants.
- **RNA library quality:** Mechanical fragmentation yields consistent insert sizes, with residual rRNA (<1%) supporting improved gene expression analysis (higher exonic read content).
- **Fusion sensitivity:** Complete detection of engineered gene fusions and splice variants is achieved at matched sequencing depth.
- **Translational utility:** This complete workflow enables robust, high-fidelity multiomic profiling for cancer research and molecular discovery applications.
- **Efficient end-to-end workflow:** Automated co-extraction and library preparation can be completed in ~18–20 hours.

References

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