An Optimized WGS Workflow for FFPE Samples: Enabling High-Confidence Variant Detection for MRD Surveillance

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Introduction

NGS Library Prep: Challenges & Solution

- **FFPE Challenges:** FFPE-derived DNA presents pre-analytical challenges for WGS-based MRD surveillance, especially across relevant genomic regions. These limitations produce non-uniform coverage and reduce variant calling sensitivity and precision[1].
- **Our Solution:** An integrated workflow of the truXTRAC® FFPE SMART Solution on the Hamilton Sonication STAR™ and truCOVER® DNA WGS Library Preparation addresses these challenges by improving input DNA quality, maximizing usable data, and ensuring reliable coverage and variant detection.

Methods

- **Samples:** 15 µM FFPE GIAB scrolls (HG002–HG005) (n=48) sourced from Horizon Discovery
- **FFPE Extraction:** Automated truXTRAC FFPE SMART Total NA (Sonication STAR) vs. Supplier α DNA/RNA FFPE kit
- **Library Preparation:** truCOVER WGS Kit with Amplification. vs. Supplier A Kit (includes Amplification) (100 ng input, 8 cycles)
- **Analysis:** Illumina NovaSeq X Plus (30x coverage); analysis via CLC Genomics Workbench and hap.py tools.

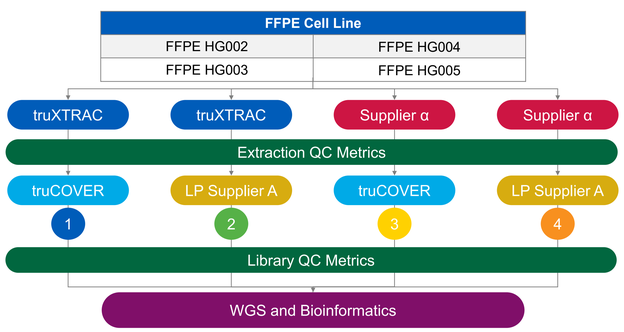

Figure 1. Experimental design for comparisons of FFPE extraction through library preparation and whole genome analysis.

Protocols and procedures are described in the truCOVER WGS Library Prep Kit product manual[3], prior publications[4,5] and in the associated Application Note[2].

Results

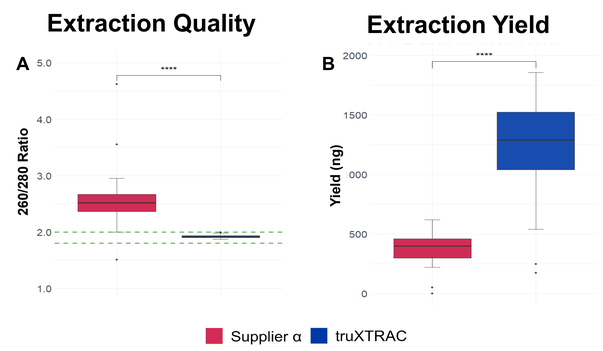

Figure 1: (**A**) Extraction Purity (260/280) and (**B**) Extraction Yield.

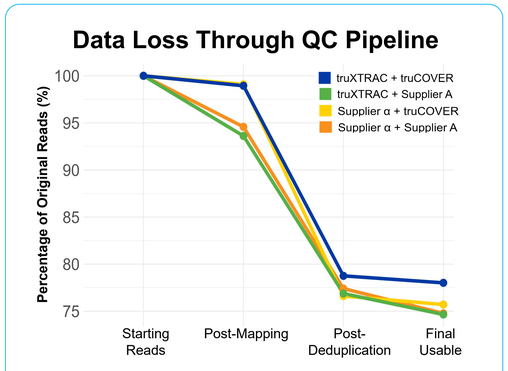

Figure 2: Usability and loss of sequencing data during initial quality assessment.

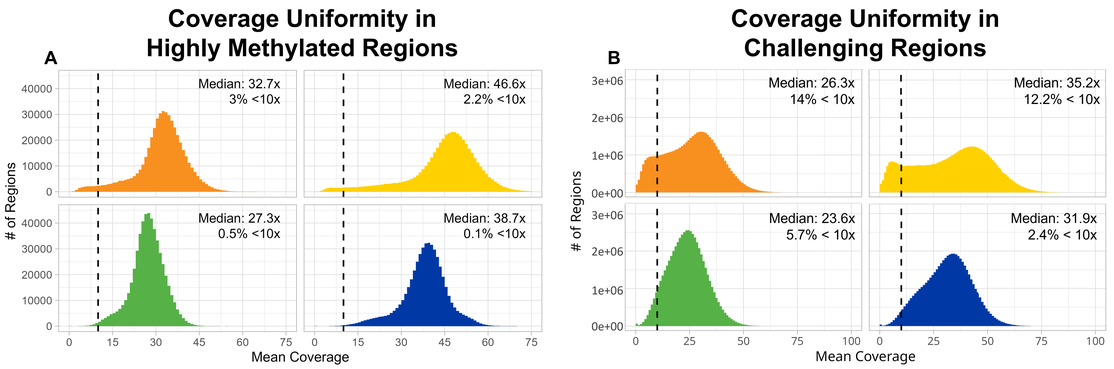

Figure 3: Uniformity of coverage in difficult and cancer-relevant genomic regions. (**A**) Highly methylated regions, identified by CHIP-Seq Bed files from ENCFF837ELM. (**B**) Complex, SVs, and All Difficult Regions, defined by GIAB.

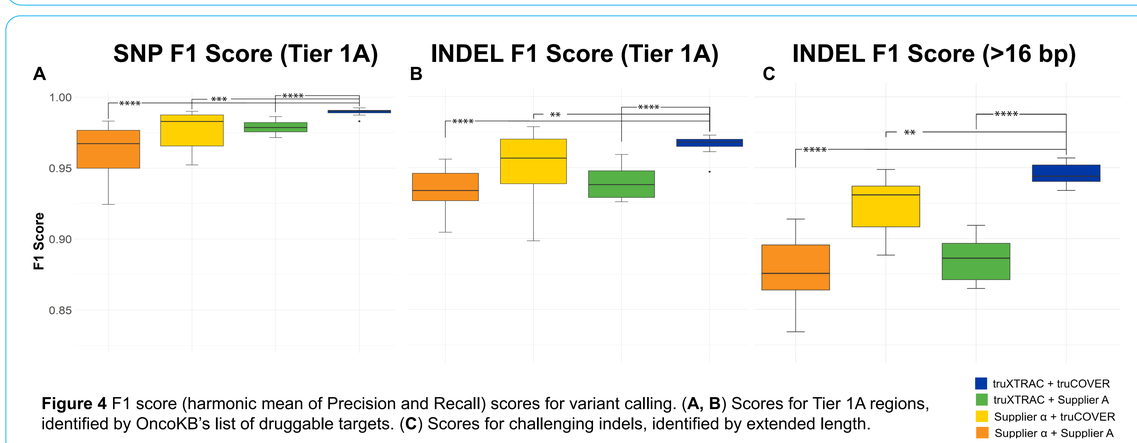

Figure 4 F1 score (harmonic mean of Precision and Recall) scores for variant calling. (**A, B**) Scores for Tier 1A regions, identified by OncoKB's list of druggable targets. (**C**) Scores for challenging indels, identified by extended length.

Conclusions

- **Scalable Workflow:** Fully automated FFPE extraction minimizes manual handling and reduces variability across samples.
- **Reduced QNS Risk:** Higher DNA recovery and improved extraction purity maximize usable input from limited or archival FFPE material.
- **Superior Read Retention:** truXTRAC + truCOVER libraries show substantially reduced data loss through mapping and deduplication QC steps.
- **Uniform Coverage Across Difficult Regions:** The combined workflow achieves superior coverage uniformity in highly methylated and structurally complex genomic regions including difficult-to-cover loci where competing workflows show marked coverage dropout.
- **High-Confidence Variant Calling:** truXTRAC + truCOVER achieves superior F1 scores for both SNPs and INDELs in Tier 1A regions, in addition to improved performance on large INDELs (>16 bp). This represents improvement on critical metrics for MRD surveillance applications.

References

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