Streamlining FFPE Workflows: Automated Sonication-Based Extraction and Optimized Library Preparation for High-Fidelity Variant Calling

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Introduction

- **FFPE Challenges:** Conventional FFPE extraction and library preparation methods as input to WGS are often low yielding, low purity, and have non-uniform coverage, leading to a reduction in usable reads and variant calling precision[1]
- **Our Solution:** An integrated workflow of the truXTRAC® FFPE SMART Solution on the Hamilton Sonication STAR ARW and truCOVER® DNA WGS Library Preparation to maximize data reliability and yield throughout the process

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

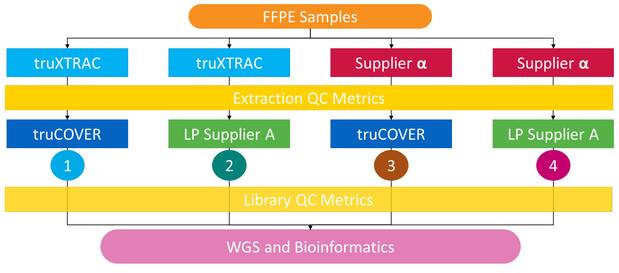

Figure 1 Experimental design for truCOVER and truXTRAC FFPE through WGS solution.

Detailed 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

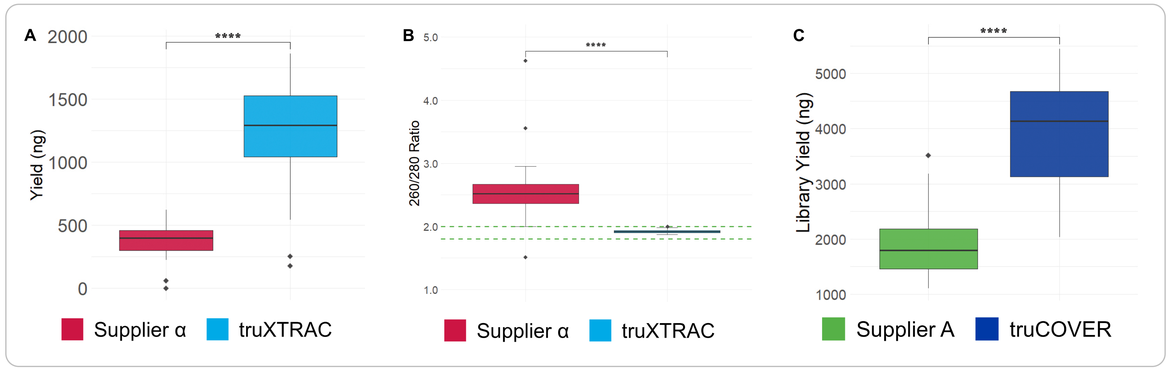

Figure 2: (**A**) FFPE Extraction Yield and (**B**) FFPE Extraction Purity (260/280 Ratio) with truXTRAC and Supplier α. (**C**) Amplified library yield from 100 ng of input after 8 cycles using truCOVER and LP Supplier A.

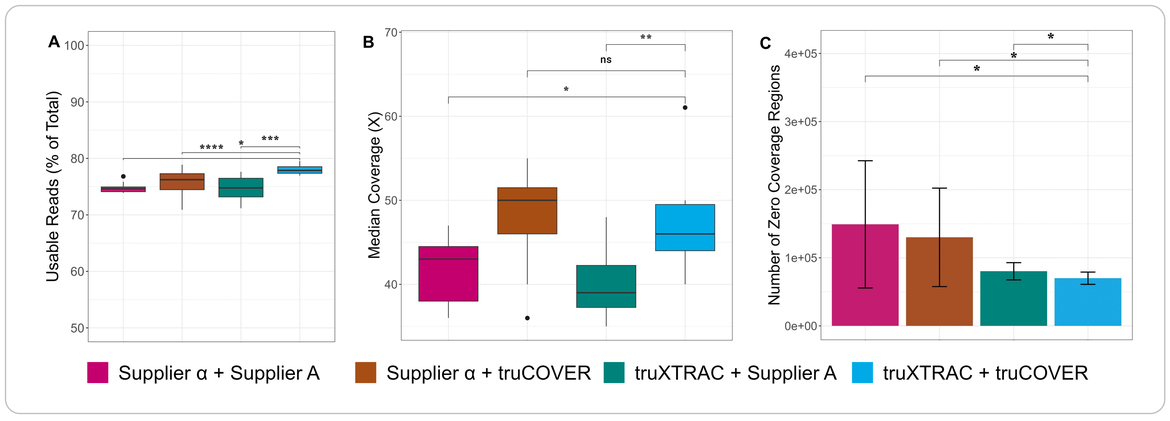

Figure 3 (**A**) Final Usable Data after bioinformatic QC (**B**) Sample Median Coverage (**C**) Regions of Zero coverage.

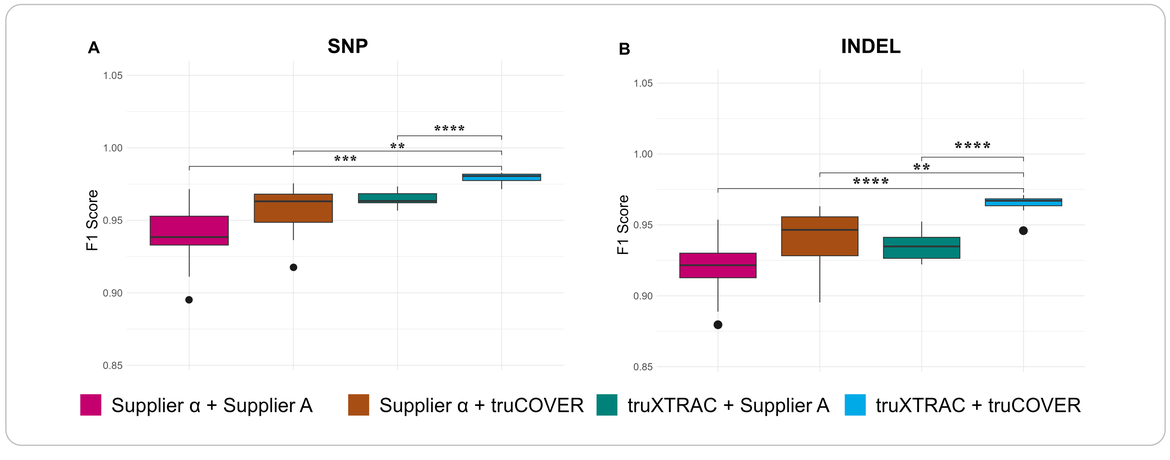

Figure 4 F1 score (harmonic mean of Precision and Recall), for (**A**) SNPs and (**B**) INDELs Libraries were prepared from 100 ng input with 8 cycles of amplification. F1 Analysis performed using *hap.py* tools from Illumina.

Conclusions

- **Scalable Workflow:** Automated extraction process with minimal manual handling
- **Reduced QNS Risk:** Higher DNA recovery and library efficiency maximize data from limited FFPE inputs
- **Enhanced Extraction Quality:** Samples extracted with truXTRAC show improved purity
- **Improved Amplification and Sequencing:** truCOVER prepared libraries show increased amplified library yield and median coverage
- **Superior Data Quality:** Significant reduction in regions of zero coverage and higher read retention
- **Superior Variant Calling:** Combining truXTRAC and truCOVER ensures high-confidence variant calling (F1 >0.95) for both SNPs and INDELs

References

1. Arreaza, G. *et al.* Pre-Analytical Considerations for Successful Next-Generation Sequencing (NGS): Challenges and Opportunities for Formalin-Fixed and Paraffin-Embedded Tumor Tissue (FFPE) Samples. International Journal of Molecular Sciences 2016, 17 (9), 1579. https://doi.org/10.3390/ijms17091579.
2. Ambavaram M, *et al.* Covaris truCOVER Library Prep Kit: A streamlined, PCR-free WGS workflow for enhanced NGS library performance and data quality. Covaris Application Note. covaris.com/wp/wp-content/uploads/resources_pdf/M020185
3. Covaris. truCOVER DNA Library Prep Kits User Manual. 2025. covaris.com/wp/wpcontent/uploads/resources_pdf/010673_User_Instructions_truCOVER_DNA.pdf
4. Amirault K., *et al.* Fully automated extraction of high-quality total nucleic acids from FFPE specimens for comprehensive genomic profiling of solid tumors. *SLAS Technology* **2025**, 31, 100252. https://doi.org/10.1016/j.slast.2025.100252.
5. Process, V., *et al.* Optimization of DNA Fragmentation Techniques to Maximize Coverage Uniformity of Clinically Relevant Genes Using Whole Genome Sequencing. Diagnostics 2025, 15 (18), 2294. https://doi.org/10.3390/diagnostics15182294.

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