High-fidelity Whole Genome Sequencing from Low-Input FFPE Samples: Enabling Accurate Tumor-Informed MRD Assay Design through Superior Variant Detection and Uniform Coverage

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Introduction

Key Benefits of the truCOVER® WGS Workflow

- Enhancing operational efficiency in sample preparation: integrated steps with or without amplification streamline the process, saving time and labor.
- Reliable DNA shearing across sample types and 2 orders of magnitude in input amounts without protocol adjustments.
- Lower total cost: fewer steps and consistent performance reduce repeats and save reagents and sequencing costs.

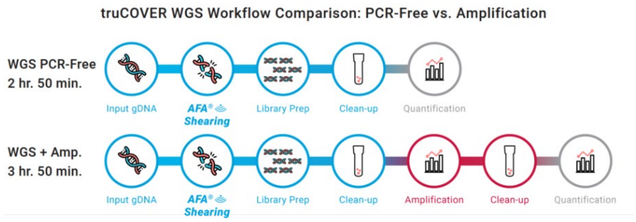


Figure 1. Overview of the truCOVER Workflow.

Methods

DNA Input and Shearing:

- High-quality gDNA (NA12878) and FFPE-derived DNA (FF12878) obtained from Coriell Institute
- FFPE DNA extracted using Covaris truXTRAC® FFPE SMART Kit on Hamilton Sonication STAR®
- Input range: 1–50 ng for library preparation
- Shearing performed using Adaptive Focused Acoustics® (AFA®) on Covaris R230 Focused-ultrasonicator

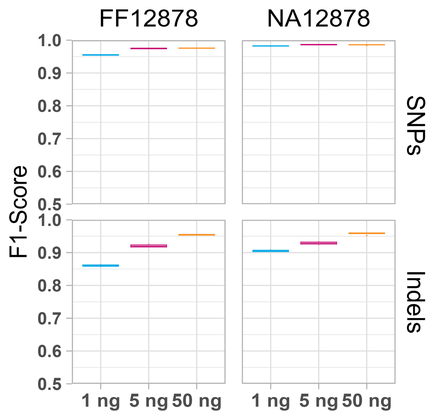
Library Preparation Workflow:

- Detailed protocols, including Covaris AFA settings, library quantification procedures[1], and workflow specifics are described in the truCOVER WGS Library Prep Kit product manual[2], prior publications[3,4] and in the associated Application Note[5]

Library Quantification and Sequencing:

- All libraries were quantified using Qubit Fluorometer & pooled equimolar based on the calculated mass.
- Pooled libraries sequenced on Illumina NovaSeq™ X Plus (~30× coverage) for performance verification.
- Sequencing data was analyzed using the CLC Genomics Workbench (Qiagen) Lightspeed Fastq to Germline Variant Pipeline.

Results



- Libraries generated from Coriell FFPE DNA (FF12878) and gDNA (NA12878)
- F1 scores evaluated for SNPs (top) and INDELs (bottom)
- High concordance observed with GIAB truth set across all inputs
- FFPE DNA performance approaches high-quality gDNA, demonstrating robust and accurate variant detection across sample types

Figure 2. High Variant Calling Accuracy from FFPE and gDNA.

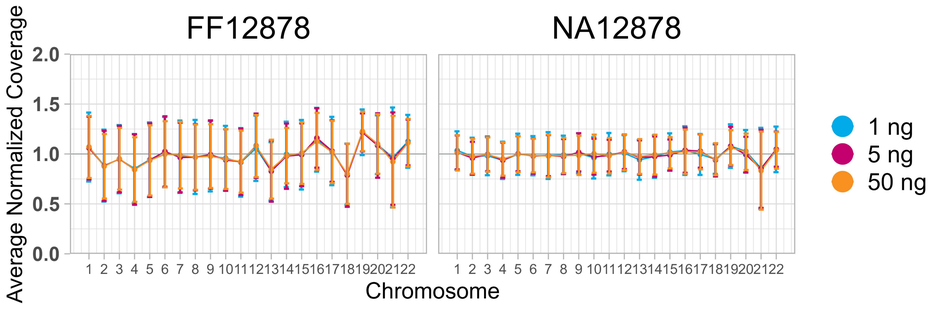
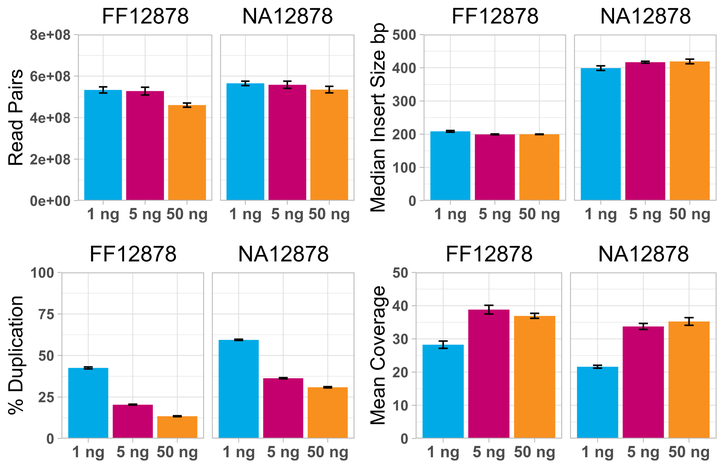


Figure 3. Uniformity of Coverage across 504 Cancer-related Genes. Coverage uniformity was evaluated using normalized gene coverage (TSO500), comparing FFPE (FF12878) and gDNA (NA12878) across autosomes with inputs from 1–50 ng.



- Robust library generation, and sequencing performance, including low-input FFPE samples
- Consistent performance across key sequencing metrics

Figure 4. Key Sequencing Metrics for FFPE and gDNA Libraries. Comparison of total read pairs (top left), median insert size (top right), percent duplication (bottom left), and mean genome coverage (bottom right) for libraries prepared across varying input amounts.

Conclusions

- **Unlock Archival Samples:** Reliably generate whole-genome sequencing data from challenging FFPE samples to enable large-scale retrospective studies.
- **Superior Variant Detection:** Achieves high F1 scores for both SNPs and INDELs from FFPE and high-quality DNA, ensuring accurate variant calling.
- **Uniform Genome Coverage**: Delivers consistent, even coverage across regions of varying GC content, reducing analytical blind spots and improving data reliability.
- **Conserve Precious Samples:** Requires minimal DNA input, preserving valuable and often irreplaceable FFPE tissue blocks for future studies.
- **Complete Workflow Solution:** Benefit from a fully validated, single-vendor workflow from FFPE DNA extraction with truXTRAC SMART Solutions to library preparation and amplification with truCOVER DNA Library Prep Kit

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