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High-Fidelity Whole-Genome Sequencing from Low-Input, Formalin-Fixed Paraffin-Embedded (FFPE) DNA using the truCOVER® WGS Library Prep with Amplification

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Covaris truCOVER WGS Library Prep Kit with Amplification Features

- **Optimized for FFPE:** A workflow specifically designed to handle low-input, degraded DNA from FFPE tissues.
- **High-Fidelity Amplification:** Utilizes a polymerase that minimizes GC bias and amplification-induced errors.
- **Low-Input Compatible:** Generates high-quality sequencing libraries from as little as 1 ng of FFPE DNA and 0.1 ng of high-quality DNA.
- **Reproducible Results:** Demonstrates high reproducibility for key library metrics across different operators and experimental days.

Covaris truCOVER WGS Library Prep Kit with Amplification Benefits

- **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.

Introduction

Formalin-fixed, paraffin-embedded (FFPE) FF12878 represents an invaluable resource for clinical and translational research, linking genomic data to long-term clinical outcomes. However, the fixation process is known to severely damage DNA, causing fragmentation, chemical modifications such as cytosine deamination, and DNA-protein cross-linking [1]. These damage types present significant challenges for next-generation sequencing (NGS), often leading to low library complexity, high duplication rates, sequencing artifacts, and biased amplification across the genome leading to confounding identification of single nucleotide variants (SNV) [2]. Consequently, genomic data derived from FFPE samples can be unreliable, complicating the accurate detection of variants, particularly in clinically relevant regions [3].

To overcome the analytical hurdles posed by compromised nucleic acid samples, the implementation of a highly optimized protocol for library construction and amplification is critical for genomics labs. Such a system must efficiently convert

low-input, chemically damaged DNA into complex, sequencing-ready libraries without introducing significant enzymatic or sequence-dependent artifacts. The truCOVER Amplification Kit, when utilized downstream of the truCOVER WGS Library Prep Kit [4] with DNA purified via the truXTRAC FFPE SMART Solution [5–7], is precisely engineered to address the challenges endemic to FFPE-derived templates. In this study, we evaluated the performance of the complete truCOVER WGS Library Prep with Amplification workflow by testing it with different input amounts and analyzing the library prep performance by looking at sequencing metrics such as uniformity of coverage and variant analysis. This study validated its capability to generate high-yield, low-bias libraries from both challenging FFPE samples and high-molecular-weight genomic DNA.

Materials and Methods

DNA Input and Shearing

High-quality genomic DNA (NA12878) and FFPE-processed NA12878 cells (FF12878) were sourced from the Coriell Institute for Medical Research (Coriell Institute, Camden, NJ, USA). DNA from the FFPE material was extracted using the Covaris truXTRAC FFPE SMART Kit with the Hamilton Sonication STAR® instrument [5–7] (Covaris, Woburn, MA, USA). DNA inputs ranging from 0.1–50 ng were used for library preparation. DNA was fragmented using Adaptive Focused Acoustics® (AFA®) on an R230 Focused-ultrasonicator. To optimize the yield of library molecules suitable for amplification, DNA fragmentation was differentially targeted according to sample integrity. High-molecular-weight genomic DNA (gDNA) from the NA12878 cell line was sheared to a median size of 500 bp, whereas the characteristically degraded DNA from formalin-fixed paraffin-embedded (FFPE) FF12878 target was at 200 bp.

Library Preparation and Amplification

Libraries were prepared using the truCOVER WGS Library Prep Kit (PN 520361KIT), followed by amplification with the truCOVER WGS Library Amplification Kit (PN 520366KIT). The number of PCR cycles was adjusted based on the DNA input amount, ranging from 4 cycles for 50 ng inputs to 12 cycles for 0.1 ng inputs.

Library Quantification and Sequencing

Final library yields were quantified using a Qubit Fluorometer, and fragment size distribution was assessed with an Agilent Fragment Analyzer. Library pools were sequenced on an Illumina NovaSeq™ X Plus instrument to a target depth of 30x coverage for performance verification (Illumina, San Diego, CA, USA). Reproducibility studies were conducted by three different operators over three days, with libraries sequenced on an Illumina NextSeq™ 2000 instrument to ~4x coverage for analysis.

Data Analysis

Sequencing data was analyzed using the CLC Genomics Workbench (Qiagen, Germantown, MD, USA) using the LightSpeed FASTQ to Germline Variants tool. Variant call files were benchmarked against the Genome in a Bottle (GIAB) HG001/NA12878 truth set to generate F1 scores for single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) [8].

Results and Discussion

Performance of the truCOVER WGS Library Prep Kit with Amplification Workflow with FFPE

The truCOVER workflow consistently produces high-quality sequencing libraries from both FFPE and high-quality gDNA , demonstrating its robustness and suitability for a range of sample types and inputs. The test setups for these two samples are summarized in ***Table 1***. The library preparation protocol demonstrated high sensitivity, facilitating robust library construction from minimal input masses of 1 ng for FFPE-derived DNA and 0.1 ng for high-quality gDNA. The resulting library yields consistently surpassed the 2 nM concentration standard required for subsequent library pooling and normalization prior to sequencing. To optimize performance across input amounts, PCR cycle numbers were adjusted accordingly. For FFPE DNA and gDNA, libraries were generated using 10 cycles at 1 ng, 8 cycles at 5 ng, 6 cycles at 10 ng, and 4 cycles at 50 ng input. The workflow also maintained precise control over target fragment sizes, with FFPE DNA libraries averaging ~200 bp while gDNA libraries averaging ~500 bp, further reflecting the consistency of AFA-enabled fragmentation across sample types.

Table 1. Comparison of the truCOVER WGS Library Prep Workflow with Amplification using FFPE derived DNA (FF12878) and Coriell gDNA (NA12878). The table summarizes minimum DNA input requirements, PCR cycle numbers (corresponding to input amounts), target fragment sizes, and resulting library yields (0.1–50 ng input for NA12878 and 1–50 ng input for FF12878).

Test Setup	FFPE DNA (FF12878)	gDNA (NA12878)
Minimum DNA Input	1 ng	0.1 ng
PCR Cycles (input DNA Amount)	-	12 cycles (0.1ng)
	10 cycles (1 ng)	10 cycles (1 ng)
	8 cycles (5 ng)	8 cycles (5 ng)
	6 cycles (10 ng)	6 cycles (10 ng)
	4 cycles (50 ng)	4 cycles (50 ng)
Target Fragment Size (bp)	200 bp	500 bp
Library Yield Range (nM)	13.0–17.3 nM	9.1–28.9 nM

Variant Calling F1 score from FFPE DNA

A primary challenge with FFPE DNA is the accurate detection of genomic variants. The truCOVER WGS Library Prep with Amplification achieves F1 scores for FFPE samples that are comparable to those from high-quality, gDNA from 5 ng and up, while it is <5% and <3% lower for INDELS and SNP at 1 ng, respectively. At 5 ng input, the F1 score for SNPs from FFPE DNA was 0.974, compared to 0.986 for gDNA. For INDELs, which are often more difficult to detect accurately in damaged samples, the F1 scores were 0.918 for FFPE and 0.928 for gDNA (***Figure 1***). This high level of harmonic mean of precision and recall demonstrates the workflow's ability to mitigate the negative effects of formalin-induced DNA damage, enabling reliable variant analysis from FFPE tissues.

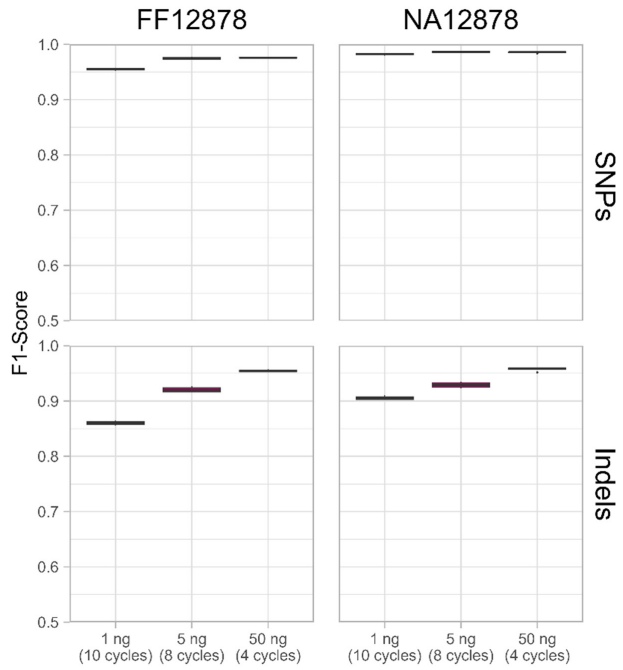

Figure 1. High Variant Calling Accuracy from FFPE and gDNA. F1 scores for INDELs (top) and SNPs (bottom) were calculated for libraries generated from Coriell gDNA-NA12878 (right) and Coriell FFPE DNA-FF12878 (left) at inputs of 1 ng (10 cycles), 5 ng (8 cycles), and 50 ng (4 cycles). The data demonstrates high concordance with the GIAB truth set for both sample types, with F1 scores from FFPE DNA approaching those of high-quality gDNA.

Unbiased Amplification and Uniform Coverage

A primary technical challenge inherent to NGS library prep and amplification for next-generation sequencing is the introduction of GC content bias leading to a non-uniform genomic coverage, which manifests as discordant read depth and might result in suboptimal representation of certain genomic intervals [9]. The truCOVER WGS Library Prep with Amplification workflow is engineered to circumvent this issue, exhibiting minimal deviation in amplification efficiency across both high-quality gDNA and degraded FFPE-derived substrates.

A critical assessment of sequencing uniformity, illustrated in ***Figure 2***, shows that the normalized coverage is consistently maintained across all 504 cancer-related genes of the Illumina® TruSight™ Oncology 500 (TSO500) panel [9], irrespective of their distribution across autosomal chromosomes. Remarkably, this high degree of normalized coverage uniformity remains centered around 1 and is not adversely impacted by varying DNA input amounts ranging from 1–50 ng, revealing the robustness of the workflow for generating reliable targeted sequencing data from both high-quality and FFPE-derived samples. DNA unbiased amplification is critical for downstream analyses requiring exhaustive genome-wide interrogation, including genomic profiling for cancer research, thereby guaranteeing the acquisition of sequencing data that accurately reflects the entire genome.

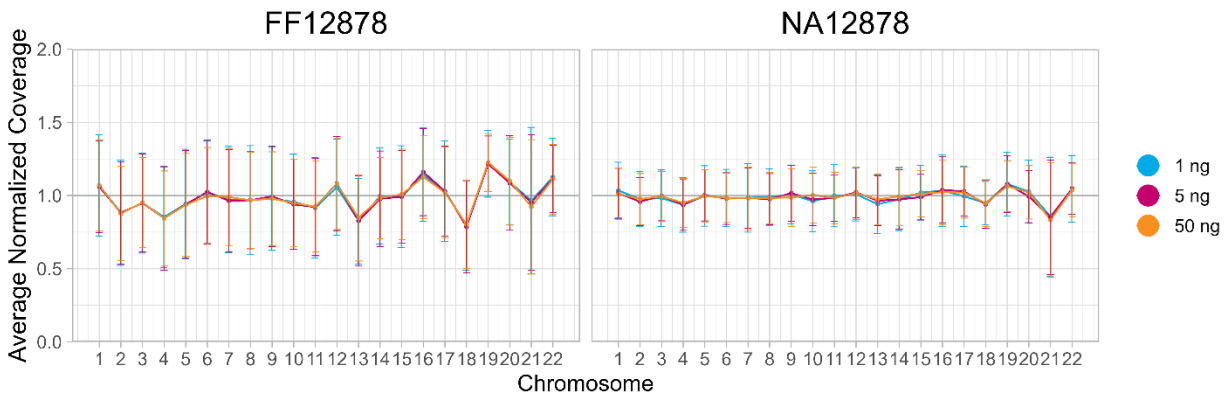

Figure 2. Uniformity of Coverage across 504 Cancer-related Genes. Coverage uniformity was assessed using the normalized coverage within genes of the CLC Genomics Workbench TSO500 region file [9]. The normalized average coverage of the genes was then plotted within each autosomal chromosome. A comparison between Coriell FFPE (FF12878) and gDNA (NA12878) is shown here. Each line plot displays an overlay of the different input amounts ranging from 1–50 ng.

Performance with Low DNA Inputs

Successful library synthesis was achieved utilizing DNA input quantities as low as 1 ng from FFPE tissues and 0.1 ng of gDNA, which reliably generated final library concentrations in excess of the 2 nM threshold necessary for creating multiplexed sequencing pools. As expected, libraries prepared from lower DNA inputs and subjected to a higher number of PCR cycles show increased duplication rates. For 5 ng input, the duplication rate for FFPE libraries was approximately 19%, which is lower than the ~34% observed for gDNA at the same input level (***Figure 3***).

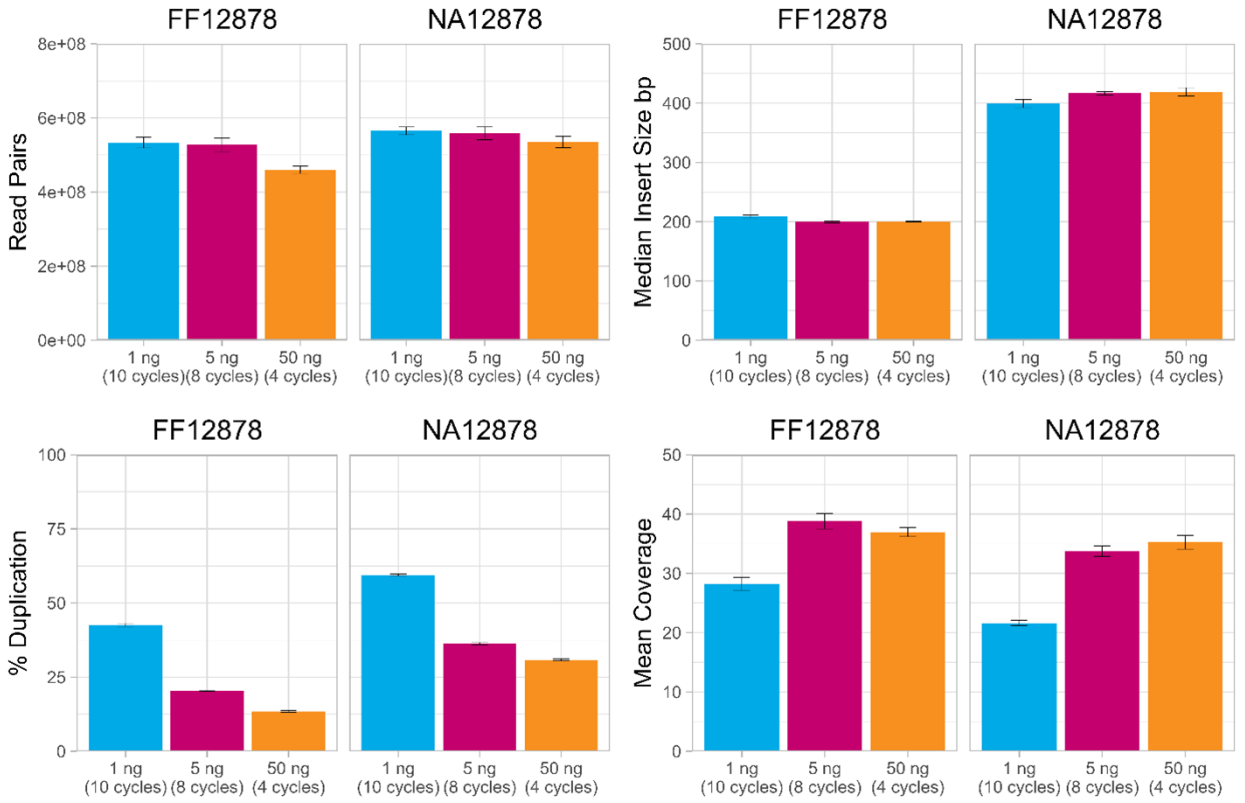

Figure 3. Key Sequencing Metrics for FFPE and gDNA Libraries. A comparison of total read pairs (top left), median insert size (top right), percent duplication (bottom left), and mean genome coverage (bottom right) for libraries prepared from Coriell gDNA (NA12878) and Coriell FFPE DNA (FF12878) at varying input amounts. The results highlight robust library generation and sequencing performance even from low-input FFPE samples.

Inter-Assay Reproducibility

To validate the workflow's robustness, an inter-operator precision study was conducted with three operators over three days. Quantitative assessment of key metrics indicated high concordance; the coefficients of variation (CV) were below 6% for modal fragment size, under 22% for library yield, and less than 16% for sequencing coverage depth across all samples (***Figure 4***). This low process-associated variability affirms the methodology's resilience to operator-introduced error, ensuring consistent performance across diverse experimental conditions.

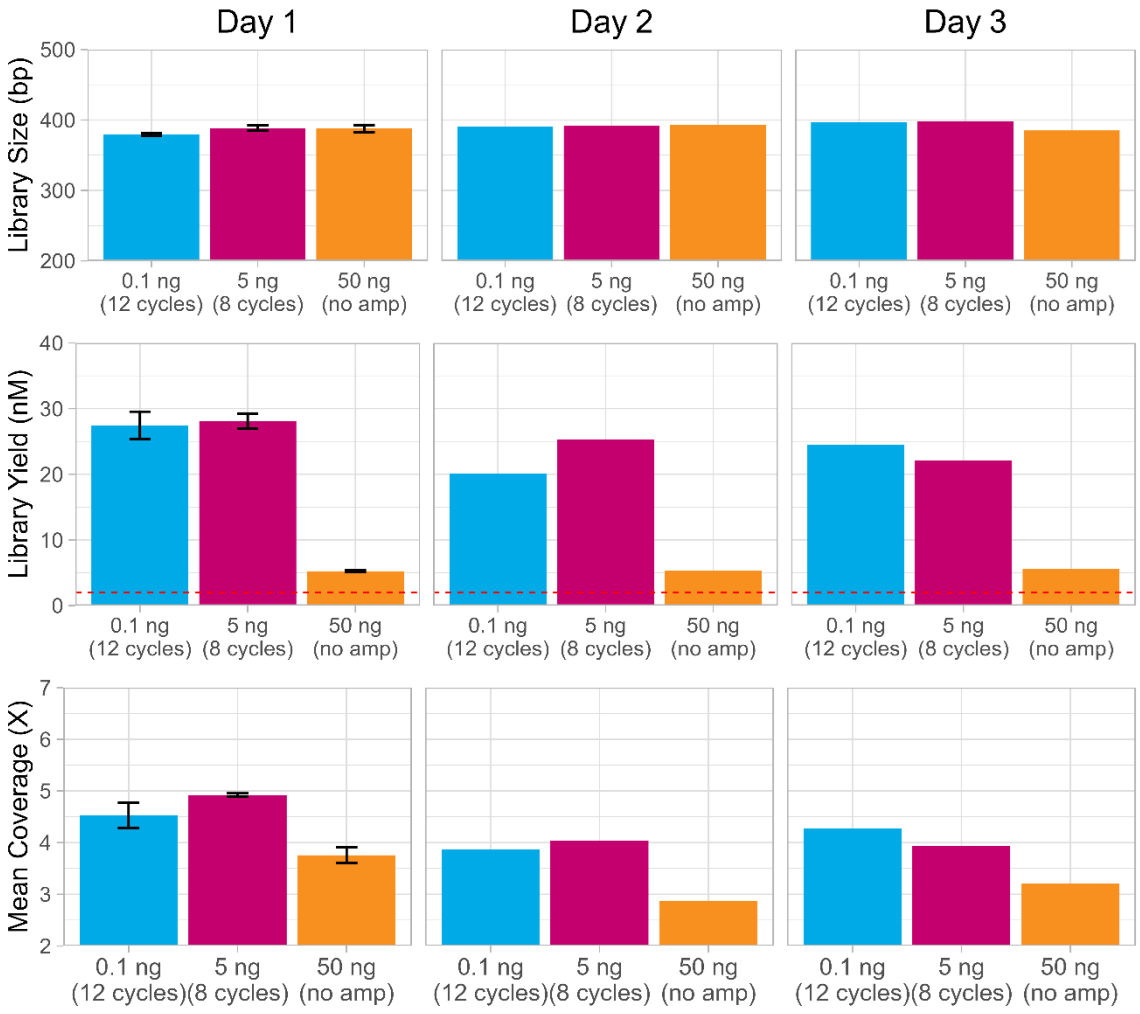

Figure 4. High Inter-Day and Inter-Operator Reproducibility for FFPE libraries. Key library QC metrics, including library size (top), library yield (middle), and mean sequencing coverage (bottom), were highly consistent across libraries prepared on three separate days by different operators for libraries prepared from Coriell FFPE DNA (FF12878). The red dashed line in the yield plot indicates the 2 nM minimum required for sequencing, a threshold met by all amplified libraries. The data demonstrates the robustness and reliability of the truCOVER WGS workflow.

Conclusion

The inherent challenges of FFPE-derived DNA have historically created significant bottlenecks in large-scale genomic studies, often requiring complex, multi-vendor workflows with variable outcomes. The data presented here demonstrate that the integrated Covaris workflow, from AFA-based extraction with truXTRAC SMART to library preparation with the truCOVER kit, provides a comprehensive and robust solution. By consolidating the most critical pre-analytical steps into a single, highly reproducible system, this workflow mitigates inter-operator variability and simplifies operational logistics.

While this study focused on WGS, the demonstrated high-fidelity amplification and low-bias nature of the libraries make them an ideal substrate for targeted sequencing applications, including whole-exome sequencing (WES) and custom enrichment panels. For reference laboratories, CROs, and academic cores, this translates directly into a reduction in sample QC failures, streamlined training, and optimized costs. Ultimately, this fully validated solution transforms the processing of FFPE tissues from an experimental challenge into a reliable, scalable methodology, empowering researchers to confidently pursue high-impact genomic discoveries from archival samples with unprecedented quality and efficiency.

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