Advancing Whole Genome Sequencing: Enhanced Library Preparation for Ultra-low DNA Inputs Using truCOVER® DNA Amplification Module

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Introduction

Key Benefits of the truCOVER WGS Workflow

- Enhancing operational efficiency in sample preparation: integrated steps with or without amplification streamlines the process, saving time and labor.
- Reliable DNA shearing across sample types and 2 orders of magnitude in input amounts without protocol adjustments.
- Easy add-on amplification process for low-input samples down to 0.1 ng with minimal amplification bias.
- Lower total cost: Fewer steps and consistent performance reduces repeats and saves reagents and sequencing cost.

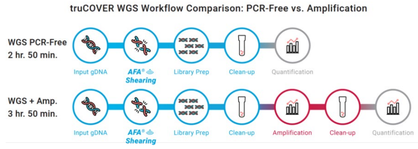

Figure A. Overview of the truCOVER Workflow.

Methods

Library Prep

- Libraries were prepared using the truCOVER WGS Library Kit and truCOVER Library Amplification Kit; input amounts varied from 0.1–50 ng (4 technical replicates). Fragmentation was done on a R230 Focused-ultrasonicator.
- DNA from Coriell (NA12878) with a GC of 41% and two bacteria strains was used as input: *Pseudomonas aeruginosa* (ATCC; 66.1% GC) and *Staphylococcus aureus* (ATCC, 32.3% GC)

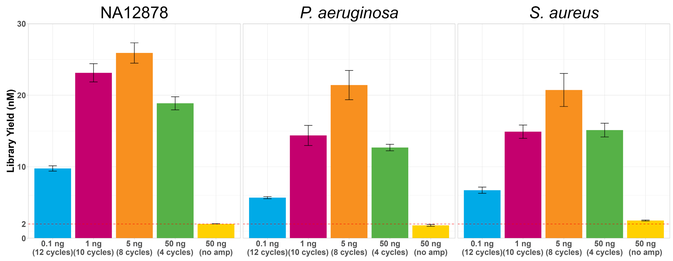

Figure B. Library yields using the truCOVER Library Kit for various input amounts. 50 ng also included with no amplification for comparison.

Sequencing

- All libraries were quantified using Qubit (Thermo Fisher) and pooled equimolar based on the calculated mass.
- Coriell and bacterial libraries were pooled together and sequenced on a NextSeq® 2000 P4 flow cell (Illumina). Bacteria were sequenced to >200x coverage and Coriell were sequenced to ~2x coverage.
- Coriell libraries were also sequenced on a NovaSeq™ X Plus 25B flow cell (Illumina) to ~30x coverage.
- Sequencing data was analyzed using the CLC Genomics® Workbench (Qiagen) Lightspeed Fastq to Germline Variant Pipeline [1].
- Reads were deduplicated using CLC's Duplicate Removal Tool which included removal based on UMI sequences.

Sequencing Results

Consistent Insert Size

- Independent of GC content, sample types and Illumina sequencer: median insert sizes were between 360–420 bp.

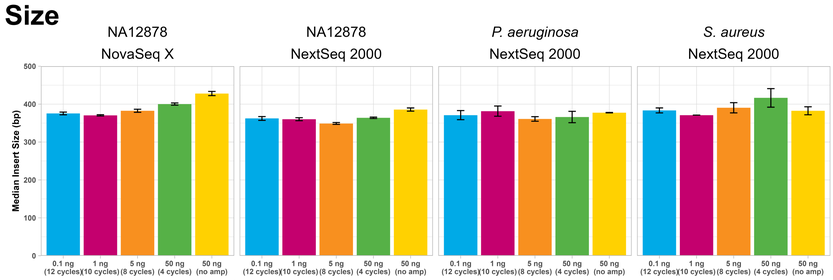

Figure C. Sequenced Insert Size from Coriell NA12878 and Bacteria on both the NextSeq 2000 and NovaSeq X.

Minimal GC Bias

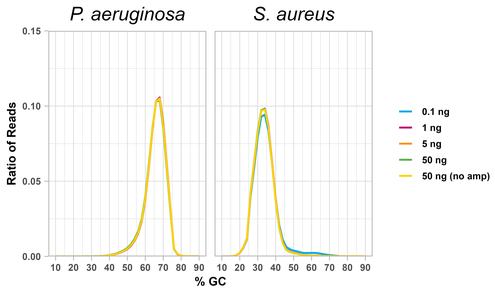

Figure D. Bacteria DNA on the NextSeq 2000. Resulting % GC of sequenced reads. The different inputs are overlapping for each bacteria.

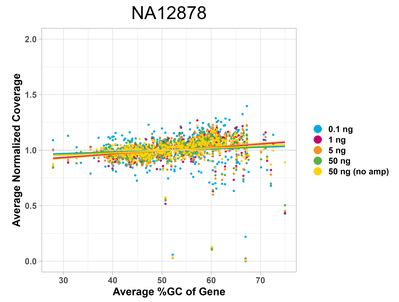

Figure E. Coriell NA12878 DNA on the NovaSeq X. GC Bias represented from the normalized coverage of clinically relevant oncogene genes. Gene regions targeted in the TruSight™ Oncology 500 (Illumina) panel were utilized to analyze the coverage across various %GC regions [2]. For each input, a best fit linear line is shown for the relationship of %GC and Coverage.

- The base composition of sequencing reads from pooled bacterial libraries remains consistent across a wide range of GC content and input amounts.
- Coverage of clinically relevant genes remains uniform across diverse GC contents and input amounts.

Uniform Coverage

- Delivers even coverage across the entire hg38 reference genome.
- High-quality coverage is maintained even when using different input amounts.

Input (ng)	Average Region Coverage	%CV Region Coverage
0.1	7.7	24.0
1	27.2	22.9
5	34.0	22.7
50	28.7	22.6
50 (no amp)	31.7	22.4

Table 1. Window region coverage metrics from the data shown in Figure F.

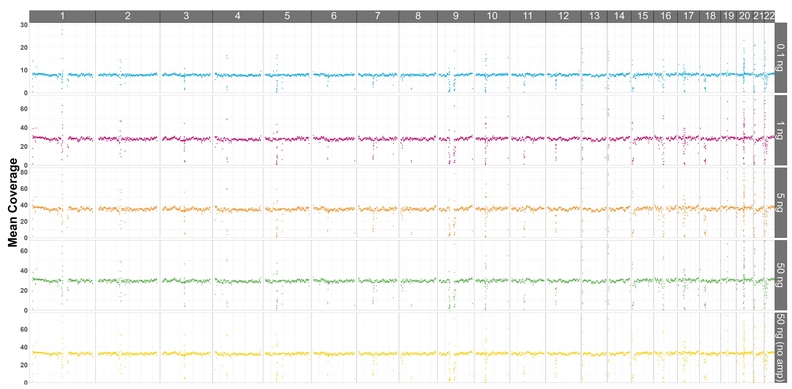

Figure F. Coriell NA12878 DNA on the NovaSeq X. Uniform coverage was accessed using the average coverage calculated across 1 Mb windows of the hg38 reference genome.

Variant Performance Comparison

Improved Variant Detection Accuracy

- Libraries were prepared with Coriell NA12878 following competitor amplification kit user guides for 3 different input amounts. A minimum input of 1 ng was chosen based on competitor kit recommendations. Sequencing and analysis was done as described in the Methods-Sequencing section.
- F1-scores were calculated by comparing the exported VCF file from the CLC against the Genome in a Bottle v4.2.1 HG001 truth set [3] using the hap.py comparison tool [4].

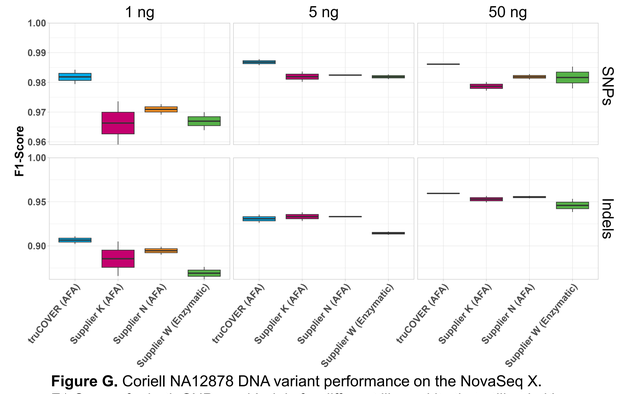

Figure G. Coriell NA12878 DNA variant performance on the NovaSeq X. F1-Scores for both SNPs and Indels for different library kits that utilized either Adaptive Focused Acoustics® (AFA®) or enzymes for fragmentation.

Results

- The truCOVER workflow offers a simplified, modular approach with minimal manual intervention and faster turnaround time, boosting lab efficiency and scalability.
- The addition of the DNA amplification module delivers linear performance regardless of sample input across varied GC content and sample types.
- The truCOVER DNA kit including amplification delivers uniform coverage for Coriell DNA which results in improved variant detection accuracy compared to competitor library kits.

References

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