Optimization of Insert Sizes for truCOVER WGS PCR-free Library with a Combination of Covaris AFA®-based Shearing and Bead-based Size Selection

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Abstract/Background

Covaris truCOVER library preparation is a PCR-free whole-genome sequencing (WGS) workflow that significantly lowers sample preparation costs by utilizing a streamlined, single-vessel approach, thereby minimizing sample loss. This method also utilizes Covaris-developed, optimized magnetic bead-cleaning reagents with enhanced library conversion rates across various sample types, resulting in improved genome coverage, variant calling accuracy, and sample concordance. In a recent collaboration with the Dana-Farber Cancer Institute to evaluate WGS library prep methods, truCOVER demonstrated reduced sample preparation turnaround time and lowered quality control costs without compromising overall NGS data quality.

The Simplest and Fastest Library Prep Workflow

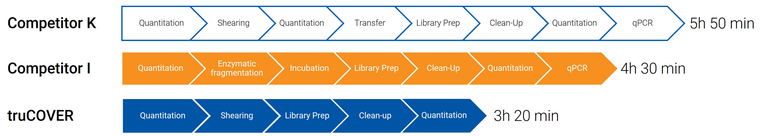


Figure 1. The Covaris truCOVER single-vessel workflow simplifies the sample preparation process compared to competitor methods.

Key Features of truCOVER Library Prep:

- Offers a faster, single-vessel workflow, eliminating loss and reducing consumable waste
- Optimized for DNA isolated from blood, saliva, and FFPE tissues
- Starting inputs of 50–500 ng in 50 µL
- Configured to result in >370 bp mapped insert sizes (Saliva & Blood DNA)

Optimization of Insert Sizes for WGS Using Covaris Adaptive Focused Acoustics® (AFA®) Shearing and Bead-Based Size Selection

A key challenge with Illumina® sequencing technology is that library insert sizes can be affected during cluster generation. In this process, libraries are distributed onto the flow cell surface and amplified, with shorter fragments clustering and amplifying preferentially. This results in a discrepancy between the library insert size determined by electrophoresis and the actual sequenced insert size.

To address these challenges, we performed sequential experiments using Coriell DNA samples, sheared to a target fragment size of 350 bp with the R230 Focused-ultrasonicator. The libraries were prepared and subjected to an optimized final clean-up step using various magnetic bead ratios, followed by sequencing on an Illumina MiSeq®. Our results, shown in ***Figures 2 and 3***, demonstrate that the dual magnetic bead-based clean-up effectively removes adapter dimers and shorter DNA fragments. This process significantly reduces the preferential amplification of smaller inserts, improving insert size accuracy on Illumina platforms.

Comparison of Initial Fragment Sizes Post-Shearing and Sequenced Insert Sizes

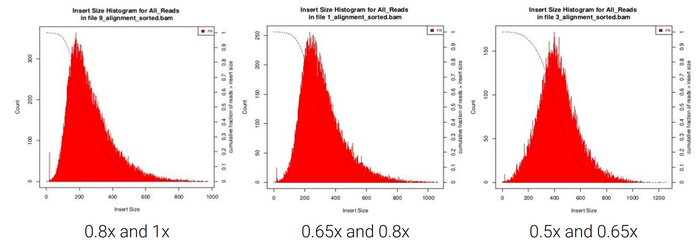


Figure 2. Histogram of the insert sizes. The Beckman Coulter, Inc.® SPRISelect® volume ratios are indicated below each histogram. With lower SPRISelect-to-sample ratios, which means more stringent size selections, the insert size distributions shift towards larger sizes.

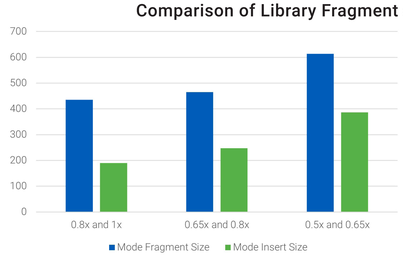
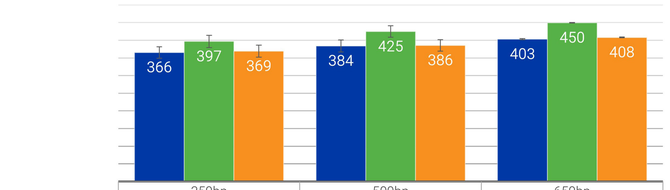


Figure 3. The bar graph illustrates the modes of size distributions for library fragments, as measured by a fragment analyzer (including adapters). Each bar represents the average size from two technical replicates, providing a comparative analysis of fragment sizes before and after sequencing.

Insert Size Bias: Controlling Insert Size Independent of Illumina Sequencer (Average Median Insert Size using Picard)



	350bp	500bp	650bp
MiSeq	366	384	403
NovaSeq 6000	397	425	450
NovaSeq X Plus	369	386	408

Figure 4. Average median insert sizes using Picard. The truCOVER protocol demonstrated high accuracy and precision in maintaining consistent insert sizes across different Illumina platforms, including the MiSeq, NovaSeq® 6000, and NovaSeq X Plus sequencers.

DNA Library Conversion and Recovery Comparison

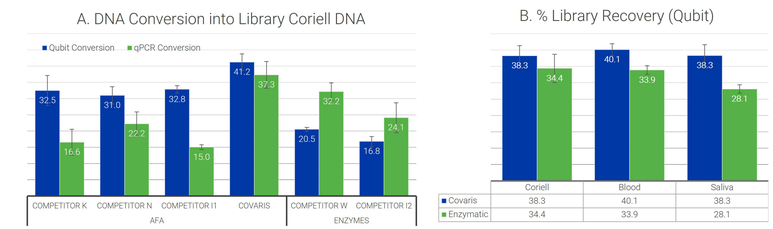


Figure 5. *A* - Illustrates DNA conversion efficiency into libraries, while *B* represents the percentage of library recovery. Covaris-based shearing consistently showed a 10–50% higher library conversion efficiency compared to competitor protocols using enzymatic fragmentation. Additionally, library recovery was significantly higher with Covaris shearing across various sample types, highlighting the robustness of mechanical shearing over enzymatic methods.

Variant Calling: High Concordance Across Sample Types

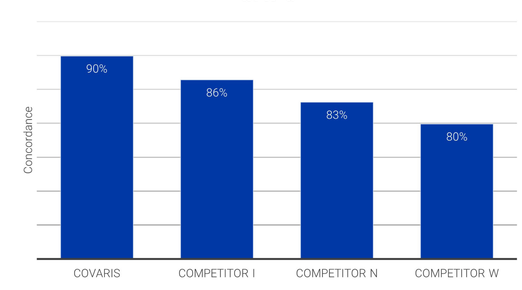


Figure 6. The bar graph illustrates variant calling analysis, showing high concordance between blood and saliva DNA samples from the same donor. This consistency ensures reliable and accurate detection of variants, outperforming competitor kits in maintaining precision across different sample types.

Conclusions and Perspectives

Covaris Adaptive Focused Acoustics (AFA) Technology, integrated with truCOVER library preparation, offers a reliable and reproducible approach for PCR-free whole genome sequencing (WGS) library preparation. This method ensures high-quality sequencing data while providing a faster, single-tube workflow. Key advantages include:

- Workflow Process: One protocol for all sample types
- Sample Compatibility: Independent of sample concentration
- Optimized Shearing: Superior control of the insert size
- Superior concordance across samples

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