Covaris truCOVER Library Prep Kit: A Streamlined PCR-Free WGS Workflow for Enhanced NGS Library Performance and Data Quality

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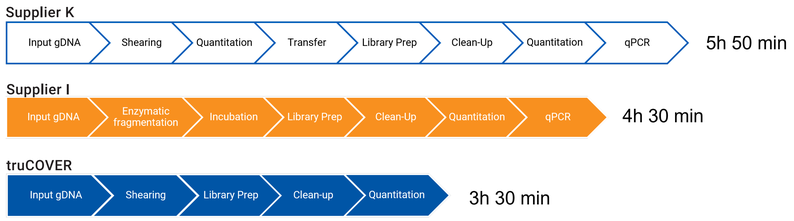
Abstract

Whole-genome sequencing (WGS) has become an indispensable tool in genomic research and clinical diagnostics, offering unprecedented insights into the underlying mechanisms of genetic diseases, cancer evolution, pharmacogenetics and pharmacology [1]. As WGS increasingly transitions from research to routine clinical use, optimization of workflows for precision, scalability, and efficiency is essential. Addressing key challenges - including inconsistency in DNA fragmentation precision, suboptimal library conversion rates, cumbersome optimization to address sample input variation and its resulting sequencing biases – is essential for ensuring high-quality sequencing data. To this end, Covaris recently launched the truCOVER WGS PCR-free Library Prep Kit, a unique solution designed to streamline the whole-genome sequencing (WGS) library preparation process. This innovative, single-vessel workflow integrates Covaris Adaptive Focused Acoustics® (AFA®)-based DNA shearing, followed by end-repair and adapter ligation, and adds a post-library size selection [2] that results in optimized fragment sizes. By optimizing fragment sizes for sequencing, this approach significantly reduces workflow complexity and turnaround time from sample to ready-to-pool libraries while lowering overall costs. The truCOVER library prep kit consistently delivers superior library conversion rates across diverse sample types, Coriell NA12878 and DNA isolated from blood and saliva. Sequencing analysis conducted in-house and in collaboration with leading institutions like the Dana-Farber Cancer Institute demonstrated improved genome coverage and enhanced variant detection accuracy with the Covaris workflow as compared to leading competitor workflows [3].

The truCOVER Workflow Provides High-Quality Performance by Ensuring:

- Compatibility with diverse DNA sources and concentrations
- Sample type flexibility
- Precise and tunable DNA fragmentation
- Consistent insert sizes during sequencing
- Accurate and simplified DNA quantification
- Pooling using mass to ensure balanced representation in sequencing
- Uniform coverage independent of GC content



Addressing Operational Efficiency: Lot Variation, Ability to Work with Multiple Sample Input Types

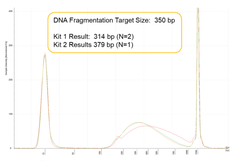
Challenges with Enzymatic Fragmentation:

- Lot-to-lot variability, which can significantly impact operational efficiency and performance
- Achieving consistent fragment size across multiple sample types

The Covaris Solution:

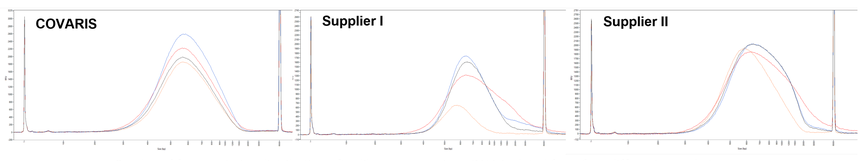
- AFA-based shearing demonstrates uniform and reproducible fragmentation
- Single shearing workflow regardless of sample type. Reduces optimization time, resources, and costs

Variability in Enzymatic Library Prep Kits Across Lots



Comparison of two lots of a Supplier enzymatic library prep kit confirms lot-to-lot variability.

Library Fragment Size Homogeneity and Consistency



Overlay of fragment size distribution after library prep using gDNA isolated from four different sample types: Coriell control (Black), blood (Blue), FFPE (Red), saliva (Orange).

Optimal Fragment Size: Ensuring High Quality Performance

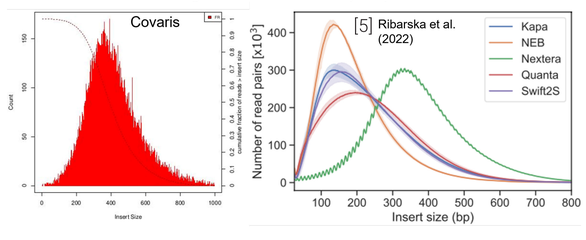
Challenges with Shorter Fragments:

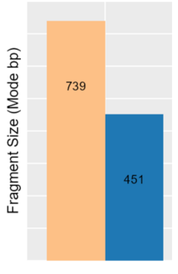
- Shorter library fragments cluster more efficiently than longer libraries [4]
- Adapter dimers can also negatively impact sequencing quality as they are smaller in size and cluster more efficiently
- Previous study (Ribarska *et al.*) with libraries created with enzymatic fragmentation resulted in ~ 150–350 bp insert size

The Covaris Solution: Covaris Shearing + Bead Purification Reagents

- Covaris shearing results in optimal fragment sizes
- Covaris bead purification reagents (0.6x/0.75x) significantly reduce the preferential amplification of smaller inserts, improving insert size accuracy on Illumina platforms
- Efficiently removes adapter dimers and shorter DNA fragments

Insert Size Distribution with Covaris Bead Purification Reagents and Other Library Prep Kits





Library Fragment and Sequenced Insert Size

Library size distribution –

- Library fragment including adapters (120 bp) with Fragment Analyzer (orange)
- Sequenced insert size (blue) without adapters.

Insert Size Bias: Managing Insert Size Independently of Illumina Sequencer

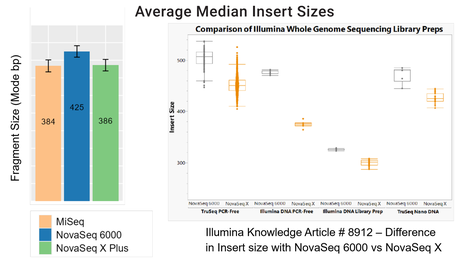
Challenges with Insert Size Consistency Across Platforms:

- Insert size representation on the NovaSeq X Series is affected by different parameters such as loading concentration, insert lengths within a library, and library cleanup [6]
- Illumina Tech Note: For insert size >200 bp, difference of ~30–110 bp observed in insert size between NovaSeq 6000 and NovaSeqX based on library prep kit used

The Covaris Solution: Optimized truCOVER workflow

- truCOVER eliminates sequencer-dependent bias toward smaller sizes seen in other library preps
- truCOVER demonstrates high accuracy and precision in maintaining consistent insert sizes across the MiSeq, NovaSeq 6000, and NovaSeq X plus

Average Median Insert Sizes



Illumina Knowledge Article # 8912 – Difference in Insert size with NovaSeq 6000 vs NovaSeq X

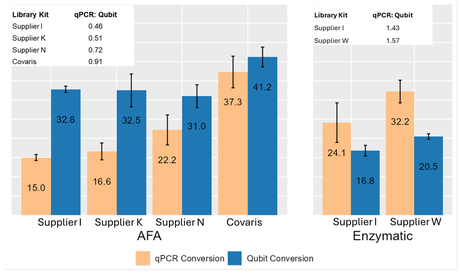
Estimating Average Median Insert Sizes using Picard Tools. DNA insert sizes measured on the MiSeq, NovaSeq 6000, and NovaSeq X Plus sequencers, evaluated from libraries prepared using the Covaris truCOVER Library Prep Kit.

Superior Library Conversion Rate Across Sample Types: Ease of Use

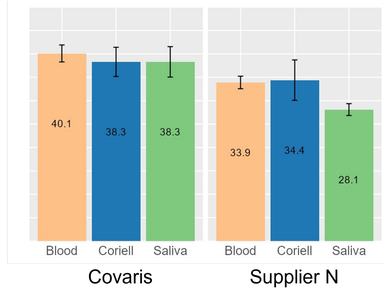
The truCOVER Workflow Offers

- Consistent library conversion rates with Qubit & qPCR: enables reliable pooling by mass, eliminating the need for qPCR-based quality-control
- Highest Conversion rates across Library prep and sample types: More sequenceable molecules with ~500 bp fragment size post shearing, lower DNA loss resulting in better representation of sequencing information

DNA Library Conversion Rates: Coriell DNA (AFA vs. Enzymatic Workflows)



DNA Library Conversion Rates: Multiple sample types (AFA vs. Enzymatic Workflows)



The bar graph illustrates library conversion rates (%), as measured by Qubit (blue) and qPCR (orange) between different AFA and enzymatic library kit workflows. Each bar represents the average conversion from four technical replicates.

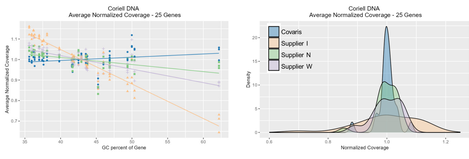
Gene Coverage Impacted by GC Content with Enzymes: Enhancing Uniformity in Fragmentation for Reliable Genomic Coverage

Challenges:

- Enzyme-based fragmentation introduces GC bias, resulting in non-uniform coverage
- Need for additional sequencing to compensate for GC bias

The Covaris Solution:

- No impact of GC content on Coverage with Covaris Library Prep kit
- Covaris library prep with AFA technology enables sequence-independent shearing, producing unbiased NGS libraries with improved coverage and reduced sequencing depth, as demonstrated across a 25-gene panel of critical cancer targets [7]



Library Kit	R^2
Covaris	0.053
Supplier I	0.906
Supplier N	0.233
Supplier W	0.532

R2 close to 0 indicates minimal correlation between GC content and uniformity of coverage.

Conclusion & Perspectives

The truCOVER WGS PCR-free Library Prep Kit, combined with Covaris AFA technology, offers streamlined NGS workflows and improves operational efficiency.

Key benefits of the truCOVER offering include:

- Flexibility: Ability to process several sample types with a wide input range
- Speed: Faster workflow with fewer touchpoints reduces turnaround time by 30%
- Cost: Simplified QC steps allows for easier and cost-effective processing
- Performance: High conversion rates, uniform coverage independent of GC content

References:

1. Rosenquist et al. (2024), Clinical Utility of Whole-Genome Sequencing in Precision Oncology. Seminars in Cancer Biology
2. Process et al. (2024), Optimization of Insert Sizes for Illumina's WGS with Covaris AFA-Based Shearing and Bead-Based Size Selection.
3. Ambavaram et.al., (2025), Covaris truCOVER Library Prep Kit: A Streamlined, PCR-Free WGS Workflow for Enhanced NGS Library Performance and Data Quality.
4. Illumina Knowledge Article #3874. How short inserts affect sequencing performance | Illumina Knowledge
5. Ribarska et al. (2022). Optimization of enzymatic fragmentation is crucial to maximize genome coverage: a comparison of library preparation methods for Illumina sequencing, BMC Genomics.
6. Illumina Knowledge Article #8912. Optimizing Library Insert Size Representation on NovaSeq X Series Instruments | Illumina Knowledge
7. Rosenthal et al (2017). Clinical testing with a panel of 25 genes associated with increased cancer risk results in a significant increase in clinically significant findings across a broad range of cancer histories. Cancer Genet.

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