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

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Introduction

Key Benefits of the truCOVER WGS PCR-free Library Prep Kit Workflow [1]:

- Enhancing operational efficiency in sample preparation: integrated steps streamline the process, saving time and labor.
- No optimization needed: DNA shearing works reliably across sample types without protocol adjustments.
- Lower total cost: Fewer steps and consistent performance reduce reagents, repeats, and sequencing waste.

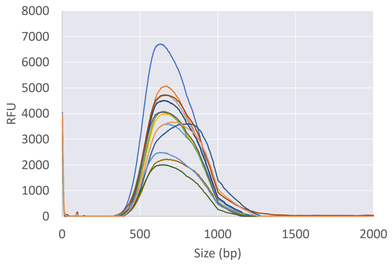

Figure 1: Electropherogram traces of DNA libraries generated from Coriell NA12878, whole blood DNA, and saliva DNA samples illustrate uniform library size distribution. All samples were processed on an R230 using the recommended settings in the product manual (PN010673).

Challenges of Library Prep

Library prep methods often suffer varying conversion efficiencies, depending on sample input amount and sample quality; especially enzymatic workflows require optimization for accurate fragmentation and additional QC steps for post-library prep pooling.

GC biases during fragmentation due to enzyme cleavage preferences may lead to under- and over-coverage of genome regions.

Methods

High-Quality Performance with a Streamlined Workflow:

- Compatibility with DNA isolated from different sources, GC-content and a wide range of input amounts (0.1 ng to 500 ng DNA)
- Precise and tunable DNA fragment size distribution
- Consistent insert sizes during sequencing
- Pooling by library mass or by equal volumes to ensure balanced coverage across libraries
- Uniform coverage independent of GC content

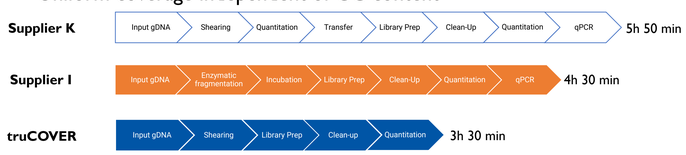

Figure 2. truCOVER WGS PCR-free Library Prep (PN520361) Workflow. Compared to leading mechanical and enzymatic methods, the truCOVER workflow simplifies library prep by combining fragmentation, end-repair, and ligation in one tube, eliminating extra clean-ups and transfers. This streamlined process reduces hands-on time and delivers up to 30% faster turnaround from shearing to sequencing-ready libraries.

Results

Enabling Pooling by Mass

- Covaris shows the highest library conversion efficiency among AFA-based kits and the best Qubit/qPCR concordance while enzymatic kits display inflated qPCR values and lower mass-based accuracy.
- truCOVER enables accurate library quantification by mass alone, with near 1:1 concordance between Qubit and qPCR, minimizing the need for additional QC steps.
- Consistent WGS coverage across Coriell, blood, and saliva samples, all exceeding the 20X depth threshold, with a grand mean of 34.8X—demonstrating robust and reproducible performance across diverse sample types pooled by mass

Unbiased Uniformity of Coverage

- Covaris library prep shows the lowest GC bias among all tested kits (R^2 = 0.053), ensuring more uniform coverage across genes with varying GC content.
- Covaris demonstrates the highest and most tightly centered normalized coverage peak at 1.0, reflecting superior uniformity and consistency across all 25 cancer-related genes [2] compared to enzymatic-based library prep kits.

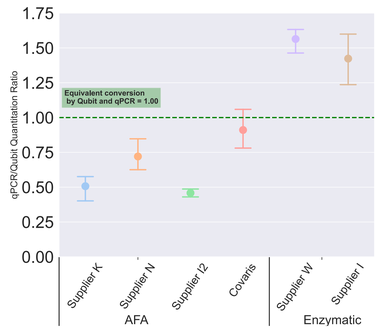

Figure 3. Comparison of qPCR to Qubit Quantification across library prep kits. Covaris' truCOVER workflow shows a qPCR to Qubit ratio near 1.0, indicating efficient conversion and accurate quantification by mass, unlike other suppliers with lower or greater ratios.

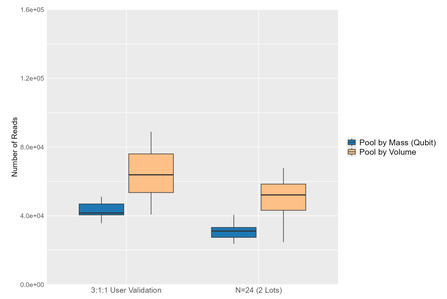

Figure 4. Library Pooling by mass (Qubit) or by Equal Volumes. Libraries were prepared with 200 ng initial gDNA input from 3 different sources (Coriell, Blood, Saliva) and pooled by equal mass or equal volumes. Number of pooled libraries were 15 (3:1:1 User Validation) and 24 (kit lot comparison), respectively. All pools were sequenced to shallow coverage on a MiSeq.

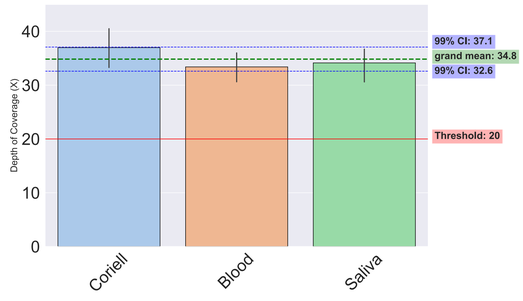

Figure 5. Depth of Coverage Across Sample Types Using truCOVER WGS Library Prep. This figure illustrates the sequencing depth achieved from DNA extracted from Coriell, blood, and saliva samples prepared using the truCOVER workflow and pooled by mass. The graph includes confidence intervals (blue dotted lines), a threshold line for minimum coverage (red solid line), and a grand mean reference (green dashed line) to assess performance consistency across varied sample types.

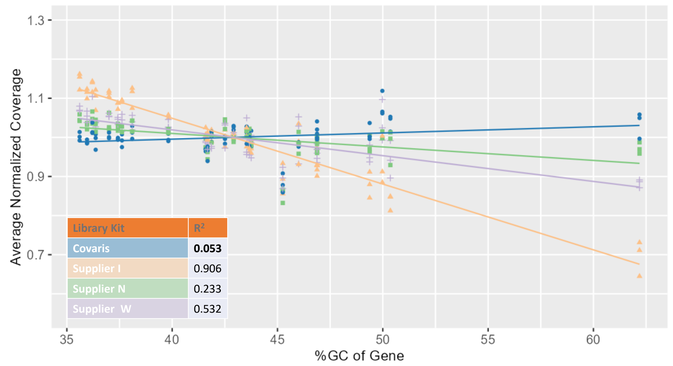

Figure 6. Average normalized coverage across 25 cancer related genes [2] with varying GC content using DNA from Coriell (NA12878). Covaris demonstrates minimal GC bias (R^2 = 0.053) compared to competitor library kits, indicating superior consistency in sequencing coverage regardless of gene composition.

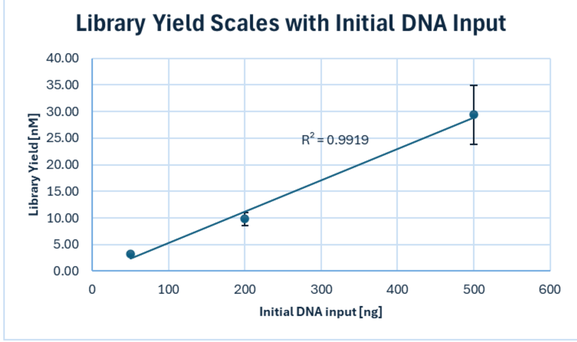

Figure 7. Library yields (mass) in relation to initial DNA input. DNA from 3 different sources (Coriell, Blood and Saliva, N=8 each) were converted to libraries. Following the manual. The plot demonstrates a very truthful conversion rate across a 10fold sample input amount. (50 ng, 200 ng and 500 ng). The truCOVER workflow settings were identical for all samples.

Conclusions

- The single-vessel truCOVER workflow reduces sample handling variability, offering a scalable solution ideal for both high-throughput labs and precision sequencing applications.
- Covaris' uniform representation across varying GC content enables more accurate variant detection and reduces the need for downstream sequencing adjustments or compensatory bioinformatics.
- The improved quantitation concordance between qPCR and Qubit allows for simplified library normalization, enabling more predictable pooling strategies and reducing the risk of uneven sample representation.

Coming soon

- Covaris is continuing to developing an integrated NGS workflow solution that provides a single-provider platform for DNA Library Prep with and without Amplification.
- This provides the foundation for a wide range of sample input amounts (3 orders of magnitude), enabling whole genome and whole exome/panel sequencing, including optimized workflows for FFPE DNA using a workflow that needs no prior optimization.
- Such Integrated Solutions will support research and clinical labs by improving operational efficiency, reducing complexity, and ensuring consistent, high-quality results across diverse sample types and applications.

References

1. Ambavaram et.al., (2025), Covaris truCOVER Library Prep Kit: A Streamlined, PCR-Free WGS Workflow for Enhanced NGS Library Performance and Data Quality.
2. 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. 218-219:58-68.

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