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Maximizing Insights from Minimal Input: High-Quality WGS Libraries from as Little as 0.1 Nanograms of DNA using the truCOVER® WGS Library Prep Kit with Amplification

Authors: Madan Ambavaram, Sameer Vasantgadkar, Sushant Khanal, Vanessa Process, Luca Beker, Alaina Villarreal, Jose Gil, Andrew Lanneville, Martina Werner, Greg Endress, Ulrich Thomann, and Eugenio Daviso
Affiliation: Covaris LLC, a PerkinElmer Company, Woburn, MA, USA 01801

truCOVER WGS Library Prep Kit with Amplification Features

- **Optimized for WGS:** Specifically designed for whole-genome sequencing (WGS) applications, especially with challenging or low-input samples.
- **Exceptional Dynamic Range:** Features a wide 500-fold input tolerance, accommodating DNA amounts from as low as 0.1 ng to as high as 50 ng for WGS. For WES, the input range can be higher.
- **Advanced Chemistry:** Employs a high-efficiency amplification system engineered to minimize bias and ensure robust performance across all input levels.

truCOVER WGS Library Prep Kit with Amplification Benefits

- **Generate High-Quality Libraries:** Reliably produce sequencing-ready libraries with reproducible fragment profiles and yields that consistently exceed standard Illumina sequencing platform requirements.
- **Achieve Superior Coverage:** Obtain high and uniform genome coverage from both low and standard DNA inputs, ensuring maximum data capture from every sample.
- **Improve Variant Detection:** Maximize the accuracy and sensitivity of your downstream analysis, leading to more confident and reliable variant detection.

Background

The ability to perform whole-genome sequencing (WGS) from samples with limited DNA input remains a significant challenge in genomics [1,2]. Conventional library preparation kits often fail to generate high-quality sequencing libraries from low nano- and sub-nanogram amounts of DNA, frequently resulting in sample loss, decreased depth of coverage, and inconclusive sequencing outcomes [3]. These difficulties are further compounded when working with microbial genomes that exhibit variable GC content, as amplification and coverage biases are particularly noticeable under low-input conditions [4].

To address these limitations, we evaluated the performance of the Covaris truCOVER WGS Library Prep Kit in combination with the truCOVER Library Amplification Kit for low-input applications using both Coriell and bacterial genomic DNA with varied GC content. In this study, libraries were prepared from as little as 0.1 ng of Coriell NA12878 genomic DNA, as well as from microbial DNA spanning a wide GC range. The results demonstrate that the truCOVER workflow provides a robust and reproducible solution for generating high-quality libraries from low-input DNA samples. The workflow also showed excellent performance independent of GC content by delivering linear amplification with the same number of amplification cycles. In addition, it consistently produced sufficient library yields (≥2 nM) for Illumina sequencing, with improved coverage and enhanced variant detection performance.

Materials and Methods

Library Preparation and Amplification using truCOVER Kits

Whole-genome sequencing libraries were prepared using truCOVER library prep (PN 520361) and amplified by the truCOVER Library Amplification Kit (PN 520366), following the user instructions [3]. Genomic DNA samples included Coriell NA12878 reference DNA and bacterial genomic DNA from *Pseudomonas aeruginosa* and *Staphylococcus aureus*. DNA was fragmented using Covaris Adaptive Focused Acoustics® (AFA®) technology, and libraries were subsequently quantified with the Qubit fluorometric system before sequencing on the Illumina NovaSeq™ X platform. Detailed protocols, including Covaris AFA settings, library quantification procedures, and workflow specifics are described in the truCOVER Library Prep Kit product manual [5] and in the associated Application Note [6].

PCR Amplification of Low-Input Libraries and Adapter Concentration

Libraries generated from low DNA inputs (0.1–50 ng) were amplified using the truCOVER DNA Library Amplification Kit (PN 520366). Amplification reactions were prepared by combining Amplification Master Mix (25 µL) with P5/P7 Primer Mix (5 µL) and adding 30 µL of this mix to 20 µL of purified libraries. PCR cycling was performed with an initial denaturation at 98°C for 45 seconds, followed by denaturation at 98°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The number of amplification cycles were selected according to the recommendations in the library product manual [5] and adjusted based on the amount of DNA input (***Table 1A***), with a final extension at 72°C for 60 seconds. To minimize amplification bias and duplication rates, the total number of cycles was kept as low as possible.

During adapter ligation, full-length indexed adapters with a 3' T overhang were added in molar excess relative to input DNA. Adapter concentration was adjusted according to DNA input (***Table 1B***). This ensured efficient ligation and minimized adapter-dimer formation, while maintaining compatibility with multiplexed sequencing workflows.

Table 1. Recommended PCR cycle numbers and adapter concentrations for the truCOVER WGS Library Prep Kit with Amplification, based on DNA input amounts. Amplification cycles and adapter concentrations were optimized to support efficient library generation across a wide range of inputs.

A. Recommended number of PCR cycles based on the input DNA.

Library Input DNA (ng)	Recommended PCR Cycles
50	4
10	6
5	8
1	10
0.5	12
0.1	12

B. Recommended adapter concentration based on the input DNA.

DNA Input (ng)	Adapter Concentration (µM)
51–500	15
25–50	7.5
10–24	3
2–9	1.5
<2	0.3

Results and Discussion

Fragment Size Distribution and Consistency

The size distribution of libraries generated using the truCOVER WGS Library Prep Kit with Amplification was evaluated using the Fragment Analyzer and ProSize® software. Smear analysis between 200 and 2500 bp yielded average fragment sizes of 688 bp and 658 bp for two independent lots (***Lot 1 and 2***), prepared using Coriell genomic DNA (***Table 2***). These sizes closely align with the expected fragment length (~640 bp), which corresponds to a target shearing size of 500–550 bp plus ~140 bp of Illumina adapter sequence. This demonstrates that the library preparation process maintains high reproducibility across different input amounts.

Table 2. Average fragment sizes generated using the truCOVER WGS Library Prep Kit with Amplification across different DNA input concentrations. Each value represents the average across four technical replicates, and the values include the average fragment size, standard deviation (SD), and coefficient of variation (%CV).

truCOVER Library Amplification Kit	Sample Type	Target DNA Input (ng)	Amplification Cycles	Average Fragment Size (bp)	SD	% CV
Lot 1(RK003482)	Coriell	0.1	12	677	18.2	2.7
		1	10	662	18.4	1.7
		5	8	709	11.9	1.9
		50	4	704	30.6	4.3
Lot 2 (RK003483)		0.1	12	655	11.0	1.7
		1	10	644	12.0	1.9
		5	8	654	19.7	3.0
		50	4	681	12.6	1.9
No Amplification Control		50	0	732	29.2	4.0

Linear Amplification

A significant advantage of using the truCOVER Amplification Kit is the capability to amplify DNA from different sample types with varied GC content as well as across a wide input range. As shown in ***Figure 1***, linear performance was demonstrated across different inputs ranging from 0.1–10 ng with 8 PCR cycles. This linear relationship was observed across diverse DNA sources with GC content from ~33% to ~66%, including Coriell NA12878 gDNA, *P. aeruginosa* DNA, and *S. aureus* DNA. The amplification performance was highly reproducible, with correlation coefficients (R^2) exceeding 0.998 for all sample types (R^2 = 0.9987 for *S. aureus*, R^2 = 0.9991 for *P. aeruginosa*, and R^2 = 0.9984 for Coriell DNA). These results confirm the kit's amplification kinetics across a wide input range, ensuring sufficient and consistent library yields even from ultra-low DNA inputs.

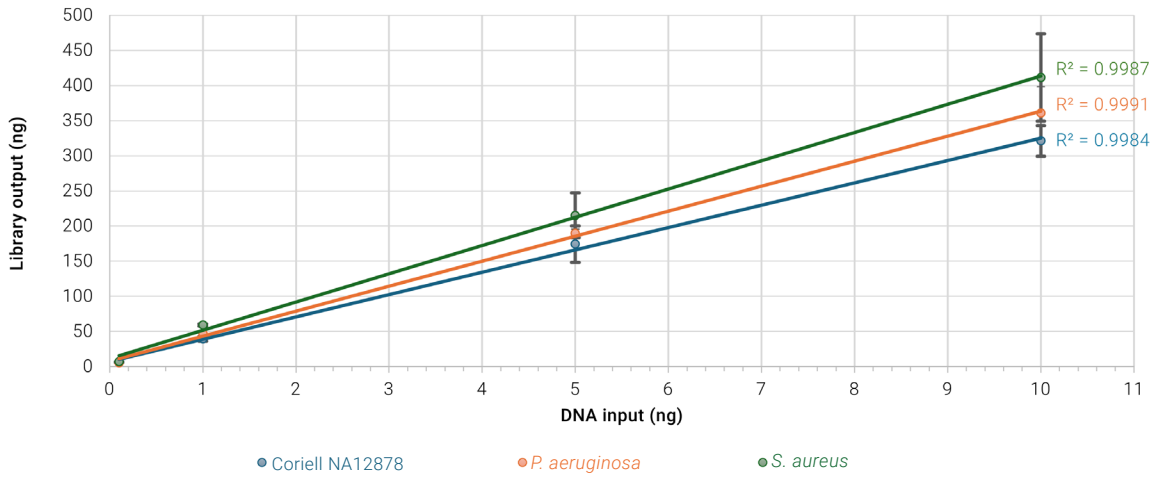

Figure 1. Linear Amplification Across Different Total DNA Input Concentrations. Scatter plot with linear regression trendlines illustrating the relationship between total DNA input and total library output after 8 cycles of PCR-amplification using truCOVER Library Prep with the truCOVER Amplification Kit. Data is presented for DNA samples from Coriell (human, ~41% GC), *P. aeruginosa* (~66% GC), and *S. aureus* (~33% GC), representing genomes with varying GC content. The error bars represent ±1 standard deviation from four replicates.

Yield Requirements for Illumina Sequencing

One critical success criterion for any library preparation workflow is its ability to generate sufficient library yield for sequencing. The truCOVER Amplification workflow consistently generated libraries that exceeded the Illumina-recommended minimum requirement of 2 nM for each input amount (***Figure 2***). At 0.1 ng input, the workflow produced an average yield of 65.1 ng (6.9 nM), while 1 ng input resulted in 80.9 ng (7.9 nM), followed by 165.6 ng (17.5 nM) at 5 ng and 113.7 ng (12.4 nM) at 50 ng input. The comparatively higher yield at 5 ng relative to 50 ng is consistent with the use of different amplification cycle numbers, as outlined in ***Table 1A***. The no-amplification (No-Amp) control at 50 ng input generated only 21.5 ng (2.3 nM), which exceeds the minimal sequencing threshold. These results highlight the ability of the truCOVER WGS Library Prep Kit with Amplification workflow to deliver robust and sufficient yields across a broad input range, enabling reliable sequencing performance even from low-input samples.

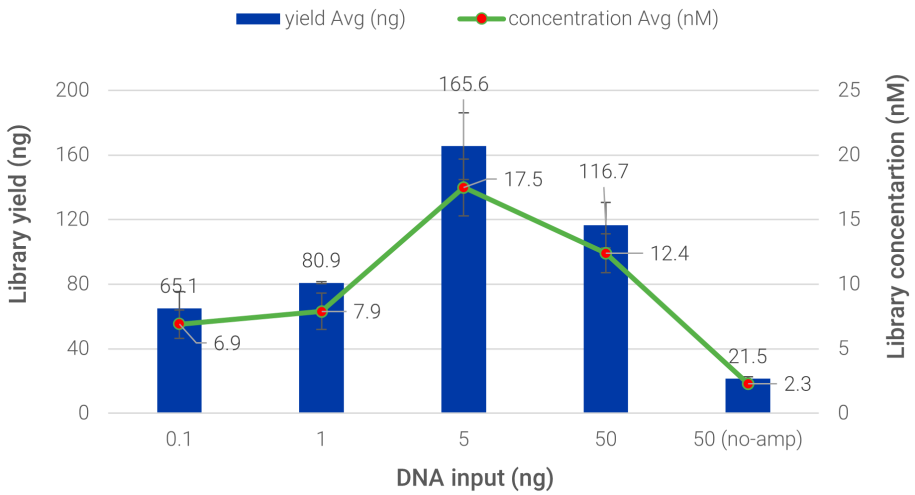

Figure 2. Bar Plot Showing Library Yield and Concentration Across a Range of Coriell NA12878 DNA Input Amounts using the truCOVER WGS Library Prep Kit with Amplification. DNA libraries were prepared from input amounts of 0.1 ng, 1 ng, 5 ng, and 50 ng of DNA using the amplification workflow, compared alongside a no-amplification (no-Amp) control at 50 ng. Average library yield (ng) and average library concentration (nM) are shown with error bars representing the standard deviation from four replicates (n=4).

Coverage and Variant Calling Accuracy

The truCOVER WGS Library Prep Kit with Amplification enhances the quality of sequencing libraries by providing robust coverage and reliable variant detection across a broad range of input DNA concentrations. As shown in ***Figure 3***, at higher inputs of 50 ng and 5 ng, the workflow demonstrated optimal performance, with coverage reaching ~41X and ~31X, respectively, and F1 scores exceeding 0.92 for INDEL s and 0.98 for SNPs. At an input of 1 ng, coverage averaged ~22X, with improved F1 scores of 0.862 for INDELs and 0.958 for SNPs. Even at the lowest input of 0.1 ng, the workflow achieved meaningful coverage of ~8X, with corresponding F1 scores of 0.607 for INDELs and 0.707 for SNPs.

By minimizing amplification bias, The truCOVER WGS Library Prep Kit with Amplification facilitates better coverage, which directly correlates with more reliable and accurate variant calls in WGS and WES studies. This outcome reduces the incidence of false negatives and positives, thereby conserving valuable time and resources in subsequent data analysis.

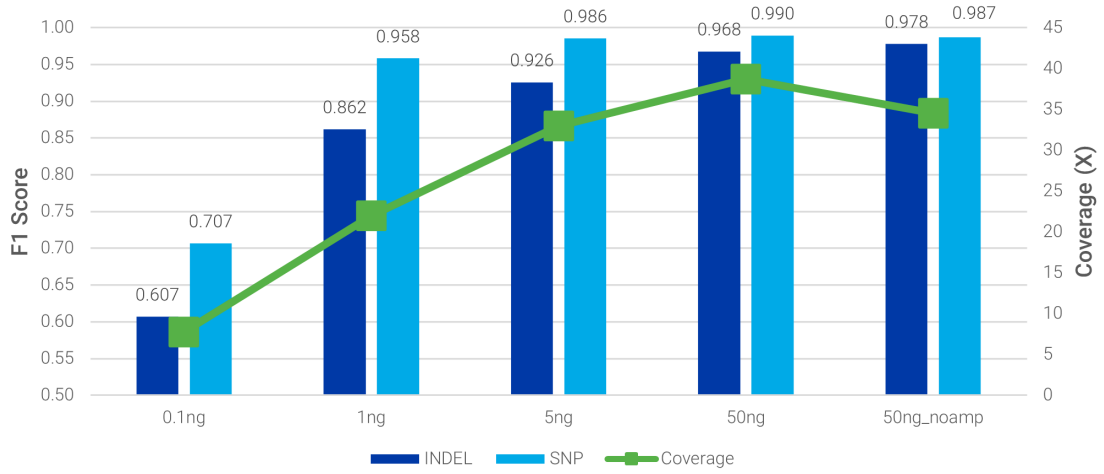

Figure 3. F1-Score and Coverage of Libraries with Varying Input DNA Concentrations. This graph demonstrates the F1 score for INDEL and SNP detection, alongside the coverage (x-fold), of equimolarly pooled libraries of Coriell DNA (NA12878). These libraries were prepared using truCOVER WGS library prep (PN 520361) and amplified by the truCOVER Amplification Kit (PN 520366), starting from different input concentrations of DNA.

Conclusion

The performance of the truCOVER WGS Library Prep Kit with Amplification directly supports critical applications requiring high-fidelity genomic analysis from limited starting material. The technical data translates into significant practical advantages, allowing researchers to overcome common bottlenecks in various scientific pipelines. For instance, in the biotech and biopharma sectors, the kit's demonstrated high-fidelity amplification from as little as 0.1 ng of DNA is impactful for drug discovery and development. Researchers can now reliably perform whole-genome screening on scarce materials, such as patient-derived xenografts, organoids, or laser-capture micro dissected tissues — critical models for drug discovery and tumor biology which often provide only minimal DNA for sequencing [7]. The demonstrated F1 scores, even at low inputs (0.607/0.707 for INDELs/SNPs at 0.1 ng, increasing to >0.92/0.98 at higher inputs), highlight the kit's ability to deliver sensitive and precise variant detection.

The ability of truCOVER WGS Library Prep Kit with Amplification to minimize GC- and PCR-related amplification bias addresses a major challenge in NGS applications that analyze samples with variable GC content. This is particularly beneficial in microbiology and metagenomics, where the genomic composition can differ dramatically between sample types. By delivering consistent performance across GC extremes (from 33–67%), the truCOVER workflow provides consistent and reliable data quality without the need for sample-specific optimization. This allows for more accurate microbial identification, characterization of antibiotic resistance genes, and comparative genomics without the need to develop sample-specific protocols, thus improving workflow efficiency and the reliability of results. Taken together, these results demonstrate that truCOVER kits offers a practical solution for a wide range of applications, including oncology, drug discovery, microbiome research, metagenomics, and clinical genomics.

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Covaris® a PerkinElmer Company

Tel: +1 781-932-3959 info@covaris.com

US: +1 781.932.3959
customerservice@covaris.com

EU: +44 (0)845 872 0100
emeacustomerservice@covaris.com

APAC: +86 137 6427 6714
APACcustomerservice@covaris.com

Applications
applicationsupport@covaris.com

Service and Support
techsupport@covaris.com

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