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When Less is More — truCOVER™ Library Amplification Kit: Empowering Next-Generation Sequencing (NGS) with Superior Amplification

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Challenges in DNA Amplification for Next-Generation Sequencing

Next-Generation Sequencing (NGS), encompassing methodologies such as Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES), has profoundly advanced genomic research and clinical diagnostics [1]. Nevertheless, a persistent bottleneck within NGS workflows pertains to the efficient and accurate amplification of DNA, particularly when dealing with limited or degraded sample inputs [2]. Conventional amplification techniques often introduce biases, leading to non-uniform coverage, preferential amplification of regions with extreme GC content, and a subsequent compromise in data quality and variant calling precision [3].

These inherent challenges present significant obstacles for researchers and clinicians:

- **Limitations with Low-Input DNA:** Obtaining high-quality sequencing data from samples containing minimal DNA quantities remains a substantial impediment. Suboptimal amplification can result in insufficient library yields, necessitating additional, time-consuming, and costly sample preparation steps or, in some instances, requiring sample re-collection [4].
- **Issues with Coverage:** Biased amplification can lead to "gaps" in coverage, where specific genomic regions are underrepresented or entirely omitted. This is critically important for WGS, where comprehensive genomic coverage is paramount, and for WES, where uniform capture of exonic regions is essential for accurate variant detection [4].
- **GC-Content Bias:** DNA sequences exhibiting extreme GC content (either exceptionally high or remarkably low) are notably challenging to amplify uniformly. This can result in skewed representation within sequencing libraries, thereby affecting the fidelity of variant calls and the overall biological interpretation [3,5].

truCOVER Library Amplification Kit: Facilitating Unbiased and Consistent NGS Outcomes

The truCOVER Amplification Kit, recommended for use with truCOVER library prep, is engineered to mitigate these challenges, providing unparalleled amplification performance for diverse NGS applications. This innovative solution ensures robust and unbiased amplification, which is crucial for acquiring high-quality data even from demanding samples.

Key Features and Benefits:

1. ***Exceptional Coverage and Enhanced Variant Calling Accuracy:*** The truCOVER Library Amplification Kit demonstrably improves the quality of sequencing libraries. As illustrated in ***Figure 1***, the kit ensures high coverage across a broad spectrum of input DNA concentrations. This translates to superior F1 scores for both INDEL and SNP detection, even with DNA inputs as low as 0.1 ng. By minimizing amplification bias, truCOVER facilitates more uniform 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.

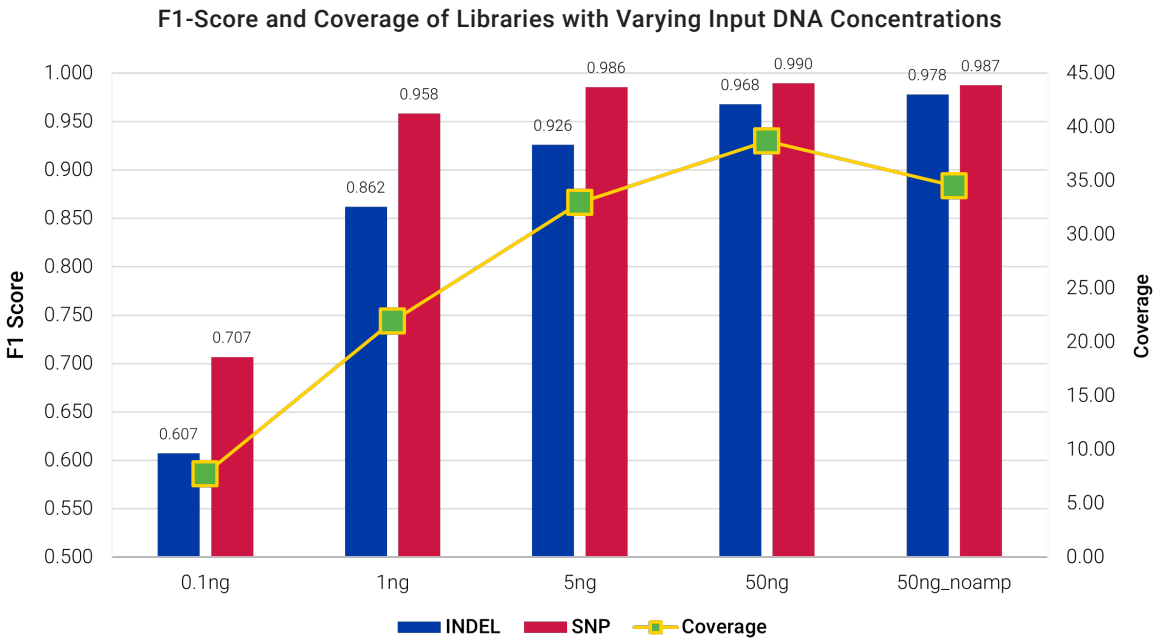

Figure 1. This graph demonstrates the F1 score for INDEL and SNP detection, alongside the coverage (x-fold), of equimolarly pooled libraries of Coriell DNA (NA12878). These libraries were prepared using truCOVER library prep and amplified by the truCOVER Amplification Kit, starting from different input concentrations of DNA.

2. ***Linear Amplification Across Diverse GC Content:*** A significant advantage of the truCOVER Library Amplification Kit is its capability to amplify DNA with remarkable linearity, irrespective of variations in GC content. ***Figure 2*** clearly depicts this linearity across various bacterial DNA samples (Coriell, *P. aeruginosa*, and *S. aureus*), which represent differing GC compositions. The high R-squared values ($R^2 \geq 0.99$) confirm the consistent and predictable amplification performance of the kit. This feature effectively eliminates GC bias, ensuring that all regions of the genome or exome are proportionally amplified, thus providing an accurate representation of the original sample and leading to more comprehensive and reliable sequencing data.

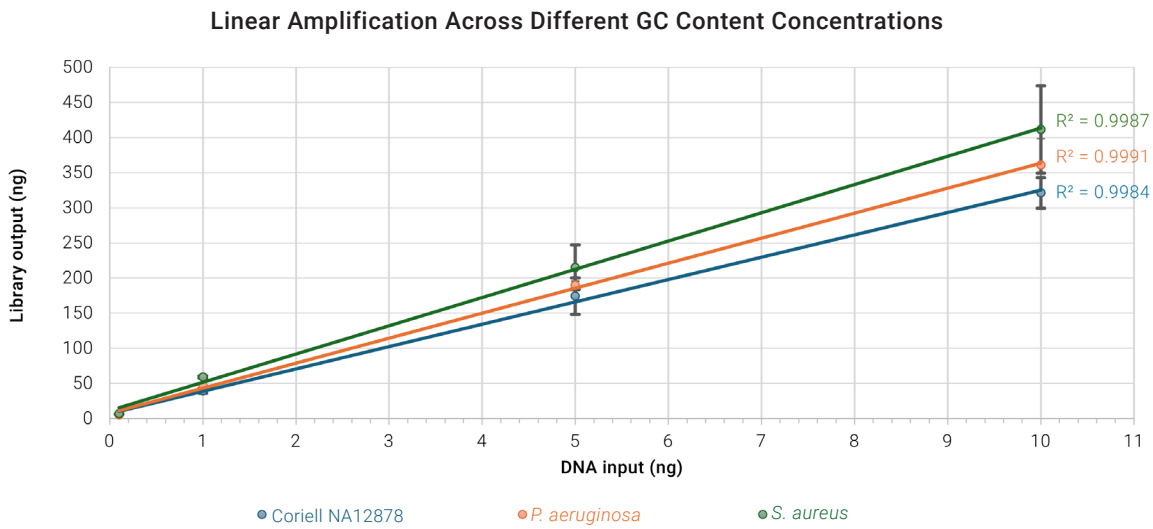

Figure 2. This plot shows the linearity of amplification (total library output vs. total DNA input) after 8x PCR-amplification using truCOVER library prep with the truCOVER Amplification Kit. Data is presented for DNA samples from Coriell (human, ~41% GC), *P. aeruginosa* (~66% GC), and *S. aureus* (~33% GC), representing varying GC content. The error bars represent ±1 standard deviation.

3. ***Streamlined Workflow and Improved Efficiency:*** The truCOVER kit is designed for user-friendliness and integrates seamlessly into existing NGS library preparation workflows (***Figure 3***). Its robust performance reduces the necessity for re-runs due to insufficient yield or biased amplification, consequently enhancing laboratory efficiency and diminishing overall sequencing costs.

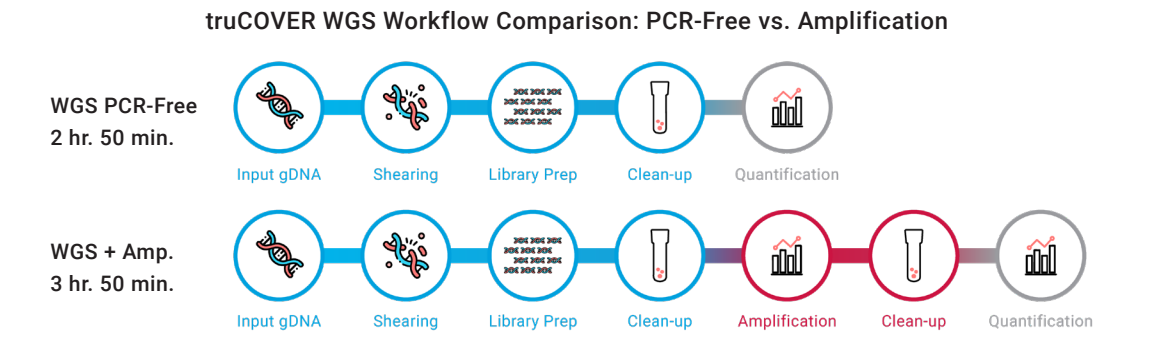

Figure 3. The diagram illustrates the comparative workflows for truCOVER Whole Genome Sequencing with and without PCR amplification, highlighting the estimated time required for each stage, from input gDNA to quantitation.

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We invite you to advance your research with the truCOVER Library Amplification Kit — For more information, visit **covaris.com/trucover-dna-library-prep-kits**

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