Twist TrueAmp Library Preparation Kit

Library preparation is a necessary, but costly and failure-prone process within the NGS workflow. Many modern NGS applications, such as WGS and target enrichment, involve using low DNA inputs from degraded sample sources while still requiring stringent sensitivity and minimal bias. The Twist TrueAmp Library Preparation Kit provides a library preparation solution that has ultra-high fidelity in AT- and GC-rich regions for rare variant detection. The kit has very high conversion rates with just 5 ng of DNA input sourced from severely degraded FFPE samples (DIN < 2.2). Overall, the kit allows users to increase the recovery of usable sequencing data from low DNA inputs.

KEY ADVANTAGES

Tunable Enzymatic Fragmentation

- Optimized fragmentation delivers larger, more uniform fragment sizes that align with the requirements of NGS platforms.

Consistently High Yields

- Achieve greater library recovery, ideal for critical applications and workflows where preserving limited or precious samples is essential.

Enhanced Uniformity

- The integration of the Twist TrueAmp Polymerase Mix improves amplification performance, supporting balanced coverage across the genome.

Wide range of input with consistent sizing and sufficient yield

Repeatable performance ensures confidence in results. When experimental conditions are held constant, the Twist TrueAmp Library Preparation Kit produces a robust, consistent DNA library fragment size across a wide range of sample inputs. Libraries generated with DNA input down to 1 ng provide sufficient mass for downstream applications and maintain consistent sizing (**Figure 1**).

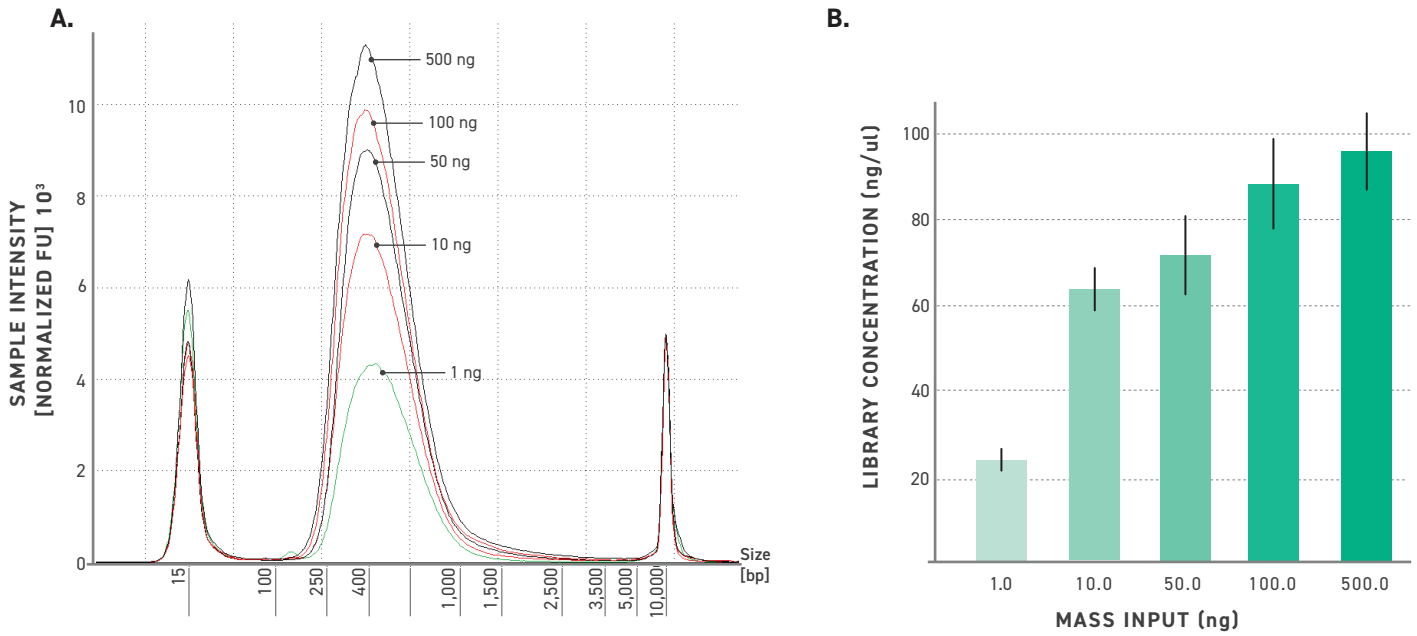

Figure 1. Reliable library size with Twist TrueAmp Library Preparation Kit, even from ultra-low inputs. 500 ng, 100 ng, 50 ng, 10 ng, and 1 ng were fragmented for various times at 32°C. 3, 5, 6, 8, and 10 cycles of PCR were utilized for amplification, respectively. Samples have been performed in duplicates for each DNA input concentration. **(A)** Electropherograms of NGS libraries generated with the Twist TrueAmp Library Preparation Kit. The overlap of the fluorescent curves demonstrates the consistency in library preparation that can be achieved from run to run. Samples were analyzed using the Agilent TapeStation D5000. **(B)** Concentration of libraries after amplification for various DNA inputs.

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Highly tunable for a wide array of applications

The Twist TrueAmp Library Preparation Kit is capable of generating libraries with a wide range of insert sizes. This ability to finely tune insert sizes allows users to tailor workflows to their specific sequencing needs (**Figure 2**).

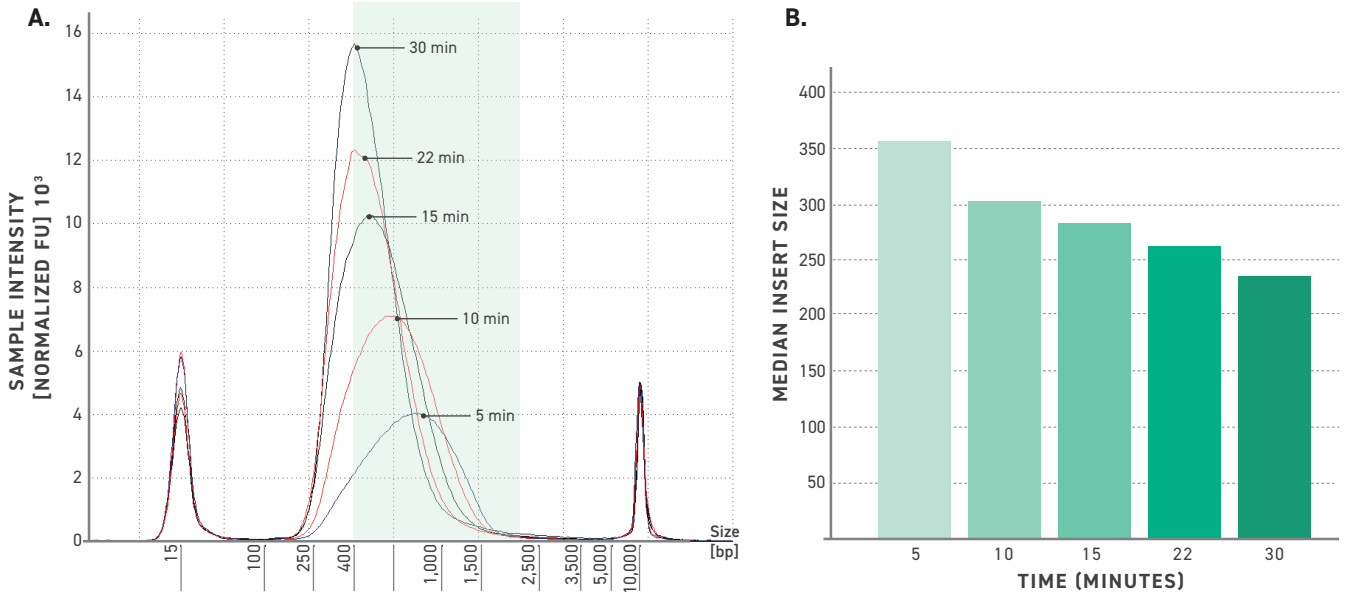
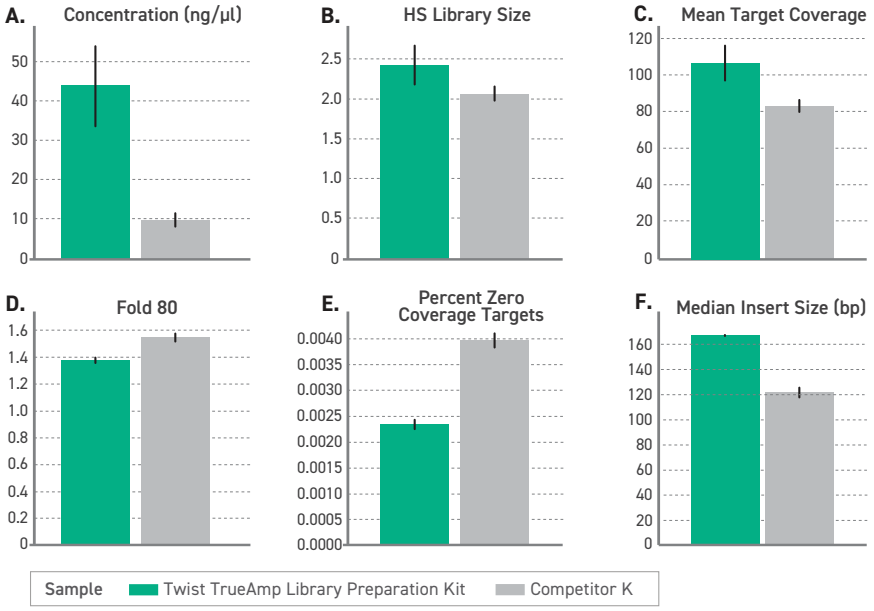


Figure 2. Tunability of Twist TrueAmp Library Preparation Kit. (A) Five electropherograms of NGS libraries generated using differing fragmentation times. 50 ng of high-quality gDNA was fragmented for various times at 32°C. 6 cycles of PCR were utilized for amplification. Samples were analyzed using the Agilent TapeStation D5000. **(B)** Median insert size vs time. 50 ng of high-quality gDNA was fragmented for various times at 32°C. Amplification was performed using 6 cycles of PCR. Samples were captured using the Twist Exome 2.0 panel and were sequenced on an Illumina NextSeq 2000. Valsera software was used to analyze Picard metrics.

Improved sequencing metrics on difficult samples

The Twist TrueAmp Library Preparation Kit produces libraries with high yields and consistent library sizes, even when using DNA from FFPE samples. With its high performance, users can achieve sufficient library quantities without relying on excessive PCR cycling, reducing the risk of sequencing bias and experimental failure. The increased yield also drives improved sequencing metrics, providing more reliable results (**Figure 3**).

Figure 3 . Performance comparison of enriched libraries with low-input FFPE degraded samples (DIN <2.2) between Twist TrueAmp Library Preparation Kit and competitor K kit, demonstrating the optimal solution for challenging sample applications. **(A)** The Twist TrueAmp Library Preparation Kit generates superior pre-capture library yield, indicative of high library construction and amplification efficiency. **(B)** The Twist TrueAmp Library Preparation Kit shows higher library complexity when compared with the competitor's kits. This allows for more unique DNA molecules that are sequenceable in the final library, reducing sequencing costs. **(C)** Achieves higher coverage. **(D)** Delivers excellent coverage uniformity, measured by a lower fold-80 base penalty. **(E)** Reduced regions with no coverage (false negative), measured by Percentage of Zero Coverage Targets. **(F)** Generates larger fragments on severely degraded samples.



All libraries were prepared following standard operating procedures using 5 ng Horizon Standard Severe HD803. Vendor-specific adapters were used to prepare the libraries, amplified with 10 cycles of PCR. Libraries were pooled and captured using the Twist CGP panel using the Twist Standard Hybridization and Wash Kit v2. All samples were sequenced at 2 x 100 on an Illumina NextSeq 2000 system and were normalized to 1000x coverage. Reads were aligned to hg38, and coverage of each exon/variant was calculated based on the BAM file generated.

Additionally, the Twist TrueAmp Library Preparation Kit reliably generates large and consistent insert sizes across a wide range of sample qualities. This enables users to process samples of varying qualities within a single, unified workflow (**Figure 4**).

To reduce read overlap and maximize efficiency, sequencing read length is typically matched to the expected library insert size. The Twist TrueAmp Library Preparation Kit's consistency helps simplify run planning—even when operational constraints require pooling lower-quality FFPE samples with higher-quality libraries on the same production-scale sequencer.

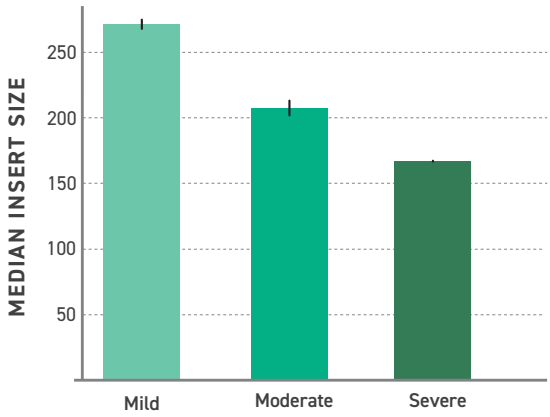


Figure 4. Library insert size from different DNA sample quality. All libraries were prepared following standard operating procedures using 5 ng of Horizon Standard Samples Mild HD798, Moderate HD799, and Severe HD803 as input. Libraries were amplified with 10 cycles of PCR. All samples were sequenced at 2 x 100 on an Illumina NextSeq 2000 system and were normalized to 1000x coverage.

Improved coverage with the new Twist TrueAmp Polymerase Mix

The Twist TrueAmp Library Preparation Kit features the newly engineered Twist TrueAmp Polymerase Mix that shows improved coverage tolerant to destabilizing uniformity enhancers, providing improved coverage of high- and low-GC regions that drop out in other polymerase solutions (**Figure 5**).

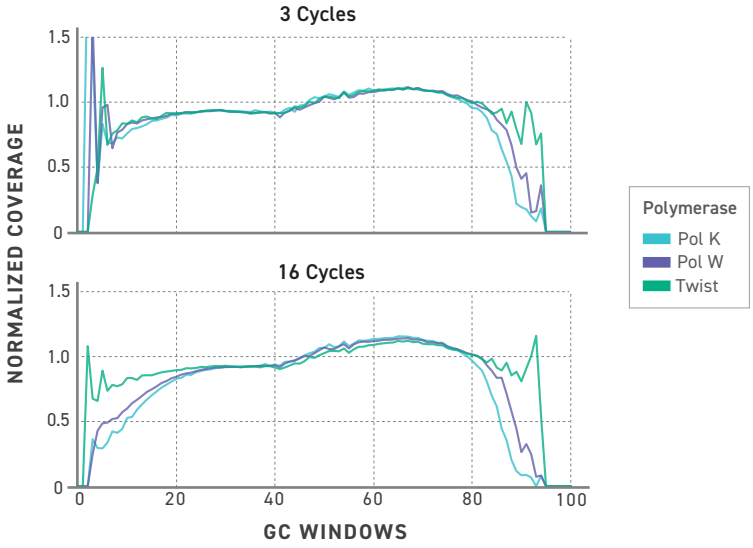


Figure 5. Normalized GC bias trace showing improved coverage of the Twist TrueAmp polymerase. Coverage Data From Illumina WGS Sequencing of a 50:50 Microbial Genomes Mix: C.difficile (AT-rich) and B. pertussis (GC-rich). Libraries were prepared with Twist TrueAmp Library Preparation Kit and amplified with different polymerases and cycles. Upper panel: Normalized coverage against GC window plots comparing polymerases at 3 cycles of PCR. Lower panel: Normalized coverage against GC window plots comparing polymerases at 16 cycles of PCR

Whole genome sequencing application (PCR-free)

For WGS on the NovaSeq X, which preferentially clusters short fragments and requires a minimum of 2 nM sample concentration for loading, higher library yield and longer insert sizes help ensure balanced clustering and more representative genome coverage. Longer inserts improve mapping accuracy, enhance detection of structural variants, and reduce bias toward short fragments, delivering more uniform and high-quality WGS data. The Twist PCR-Free WGS Library Preparation Kit enables customers to use DNA sample inputs as low as 37.5 ng for PCR-free applications (**Figure 6**). With few overlapping regions, sequencing efficiency is enhanced, and users can obtain useful data with less depth (**Figure 7**).

The Twist TrueAmp Library Preparation Kit has optimized fragmentation and ligation reagents that enhance uniformity even in PCR-free workflows. With better uniformity and low bias in coverage, users can more efficiently utilize their sequencing data (**Figure 8**).

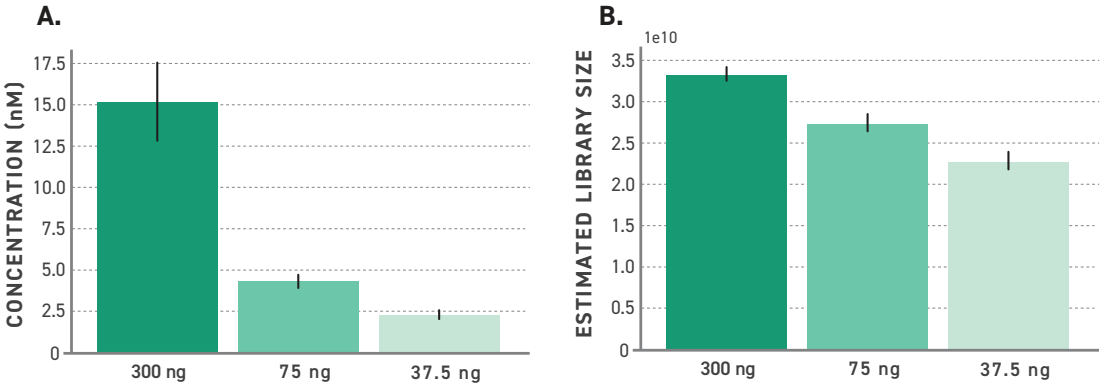


Figure 6. High library yield and estimated library size. (A) Low DNA input has usable library yield. **(B)** Library diversity is maintained even with low DNA input. Twist PCR-Free WGS Library Preparation Kit libraries were prepared using NA12878 300 ng, 75 ng, and 37.5 ng genomic DNA . Fragmentation parameters were set at 15 minutes and 25°C. Libraries were quantified by qPCR using the Kapa Library Quantification kit.

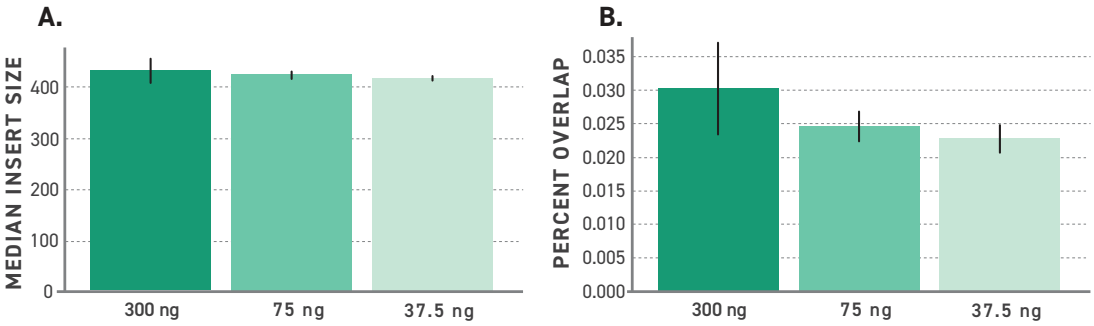


Figure 7. Twist PCR-Free WGS Library Preparation Kit insert size is consistent across samples with minimal overlap in reads. The kit produces consistently large inserts across a wide range of DNA samples. **(A)** Median Insert Size is represented for 300 ng, 75 ng, and 37.5 ng of NA12878 DNA sample input. **(B)** Percent Overlap is represented for 300 ng, 75 ng, and 37.5 ng of NA12878 sample input.

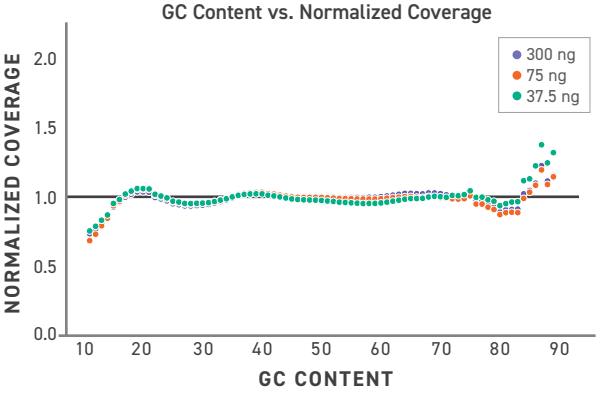


Figure 8. The Twist PCR-Free WGS Library Preparation Kit provides more consistent coverage distribution across varying GC content. The kit shows minimized GC bias even at low inputs, thereby reducing the need for additional sequencing to achieve uniform coverage and better detection of variants in the high- and low-GC content regions.

LEARN MORE

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ORDERING INFORMATION

116480 Twist TrueAmp Library Preparation, 16 Samples

116469 Twist Library Preparation Kit 1, 16 Samples
100401 Twist Library Preparation Kit 2, 16 Samples
116471 Twist TrueAmp Polymerase Mix, 16 Reactions

116481 Twist TrueAmp Library Preparation, 96 Samples

116470 Twist Library Preparation Kit 1, 96 Samples
100573 Twist Library Preparation Kit 2, 96 Samples
116472 Twist TrueAmp Polymerase Mix, 96 Reactions

128388 Twist PCR-Free WGS Library Preparation Kit, 16 Samples

116469 Twist Library Preparation Kit 1, 16 Samples
100401 Twist Library Preparation Kit 2, 16 Samples

128389 Twist PCR-Free WGS Library Preparation Kit, 96 Samples

116470 Twist Library Preparation Kit 1, 96 Samples
100573 Twist Library Preparation Kit 2, 96 Samples

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