TECH NOTE

Twist Library Preparation Enzymatic Fragmentation Kit 2.0 Demonstrates Robust Performance Compared With Alternatives

INTRODUCTION

Robust library preparation methods are foundational to the success of any next-generation sequencing (NGS) workflow. Whether performed manually or with automation, reliable preparation chemistry is the first step to ensuring data consistency across a variety of sample types, inputs, and GC content. This consistency is valuable for whole genome sequencing and is critical for samples that are being further enriched for specific regions of interest, enabling more in-depth sample analysis and significant cost savings.

Two methods for shearing input DNA are mechanical fragmentation via sonication or enzymatic fragmentation. Although enzymatic fragmentation can be preferable since it does not require additional equipment, the method can be vulnerable to confounding sequence bias without optimization. To address these challenges, Twist Bioscience offers the Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0. The kit is fully optimized with Twist's target enrichment and other modular workflow products, offering a robust and complete end-to-end solution.

This tech note highlights the performance of our Twist Library Preparation EF Kit 2.0 relative to other alternatives. Our complete solution of the Twist Library Preparation EF Kit 2.0 with target enrichment reagents clearly shows superior performance on various parameters highlighted below.

LIBRARY PREPARATION WORKFLOW

The Twist Library Preparation EF Kit 2.0 provides all the reagents needed for enzymatic gDNA fragmentation, end-repair, dA-tailing, adapter ligation, bead cleanups, and library amplification (**Figure 1**). This workflow can be easily adjusted to use a variety of Illumina TruSeq compatible adapter systems including Twist CD Index adapters, Twist Universal Adapter System, and Twist UMI Adapter System.

Twist's EF Kit 2.0 features a streamlined workflow that combines fragmentation, end repair, and ligation steps into a single-tube reaction. This minimizes pipetting steps and hands-on time, which reduces the potential for errors (**Figure 2**). The protocol can be easily automated on various platforms and allows for flexible mass input between 1 ng to 500 ng of DNA. Furthermore, by including the Equinox Library Prep Amp Mix—a high-fidelity, hot-start enzyme formulation—the EF Kit 2.0 has high efficiency even at low input masses. All these efficiencies ensure that the resulting amplified libraries are robust, high-quality, and high-yield.

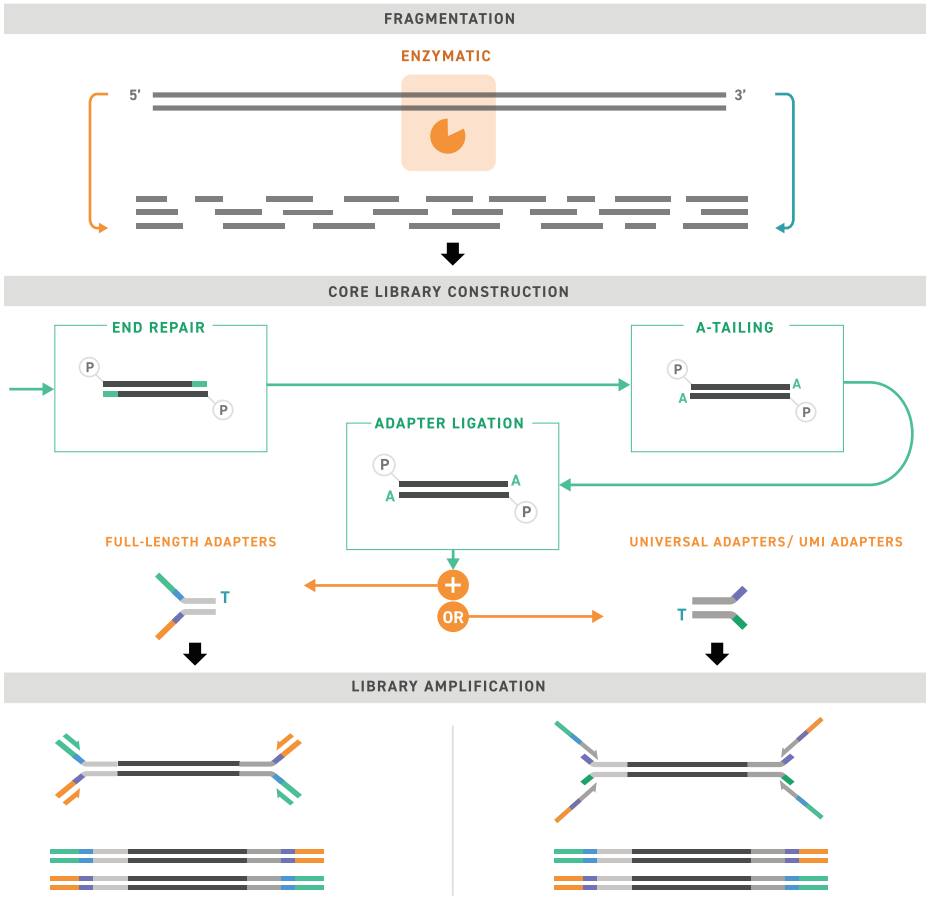


Figure 1. Twist Library Preparation EF Kit 2.0 Workflow.

TUNABILITY

The Twist EF Kit 2.0 delivers highly tunable DNA fragmentation through adjustment of both fragmentation time and temperature. Modulation across two variables provides multiple paths for optimization of manual and automated workflows. The wide range of library distributions makes the workflow compatible with a variety of applications and sequencing read lengths (**Figure 3**).

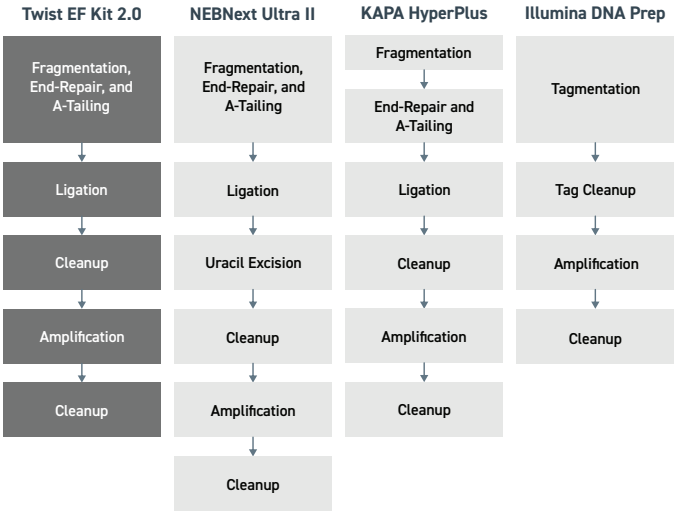


Figure 2. Workflow Comparison.

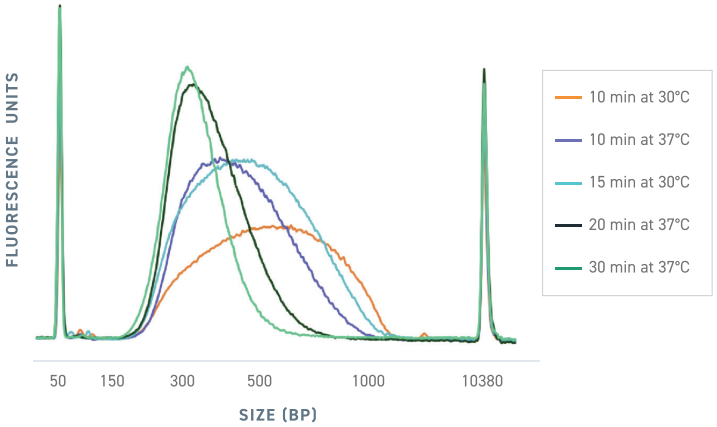


Figure 3. Insert Size Tunability. NGS libraries were generated with the Twist Library Preparation EF Kit 2.0 and Twist's Universal Adapter system. 50 ng of high-quality gDNA was fragmented for various times at 30°C and 37°C. Samples were analyzed with an Agilent DNA 7500 assay and results were overlaid in Expert 2100 software.

LIBRARY SIZE

Consistent and uniform library sizes are crucial for generating high-quality sequencing data. Typically for 2x100 bp sequencing, library insert sizes should be ~200 bp, as this size gives the best target coverage while maintaining efficiency during target enrichment and sequencing. Using protocol guidance to generate 200 bp insert libraries from multiple vendors, Twist EF Kit 2.0 generates expected insert size across a wide range of DNA mass inputs. Other protocols exhibit variable fragmentation based on mass input, leading to inconsistent libraries. With Twist EF Kit 2.0, samples of varying quantities can be run in parallel using constant conditions and generate highly reproducible library sizes (**Figure 4**).

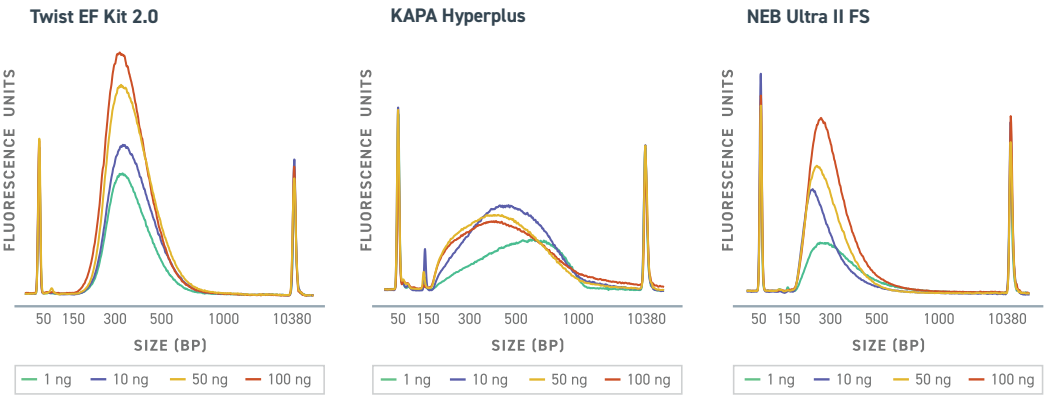
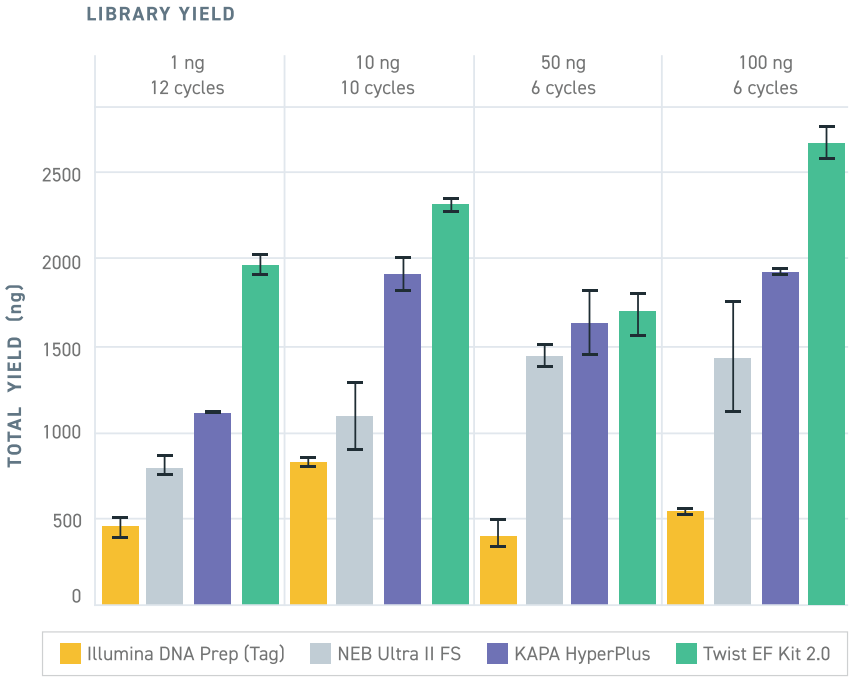


Figure 4. Average Library Size Electropherograms. Libraries were prepared with NA12878 gDNA using the input mass listed. For KAPA Hyperplus, input DNA was cleaned up with 3X beads prior to library generation, following protocol recommendations. For Twist Library Prep EF Kit 2.0, KAPA™ Hyperplus and NEBNext © Ultra II FS samples were fragmented for 20 minutes at 37°C. Samples were analyzed with an Agilent DNA 7500 assay and results were overlaid in Expert 2100 software.

LIBRARY YIELD

A key metric to determine successful generation of a library is yield. This metric shows if a reaction is performing as expected or not, and a high yield indicates good library conversion efficiency in the reaction. Obtaining high library yields is difficult with low DNA mass inputs and often requires performing additional PCR cycles. Using additional PCR cycles risks creating a biased library that does not adequately represent the original DNA sample. With the Twist EF Kit 2.0, even low mass inputs consistently generate sufficient library yield for downstream target enrichment with minimal PCR cycles. When using a range of mass inputs, the Twist EF Kit 2.0 has consistently higher library yields in comparison with other common library preparation kits (**Figure 5**).

Figure 5. Twist Library Preparation EF Kit 2.0 Produces Superior Library Yield Across a Wide Range of Mass Inputs. Libraries were prepared with NA12878 human gDNA using the input mass and PCR cycles listed. For KAPA Hyperplus, input DNA was cleaned up with 3X beads prior to library generation, following protocol recommendations. For Twist EF Kit 2.0, KAPA™ Hyperplus, and NEBNext © Ultra II FS libraries, samples were fragmented for 20 minutes at 37°C. Average library yield is reported, error bars represent the standard deviation between replicates.



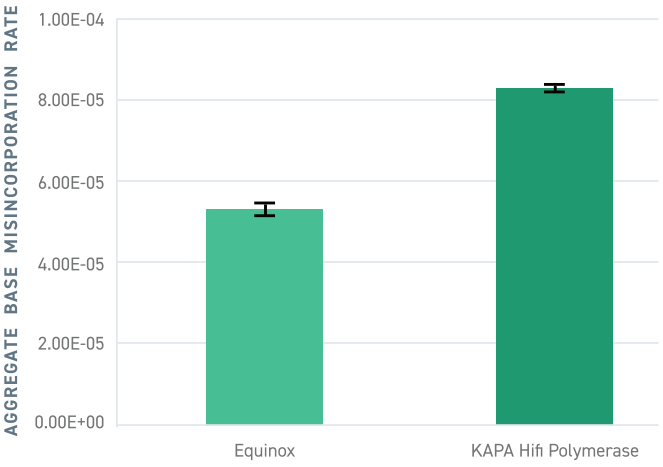
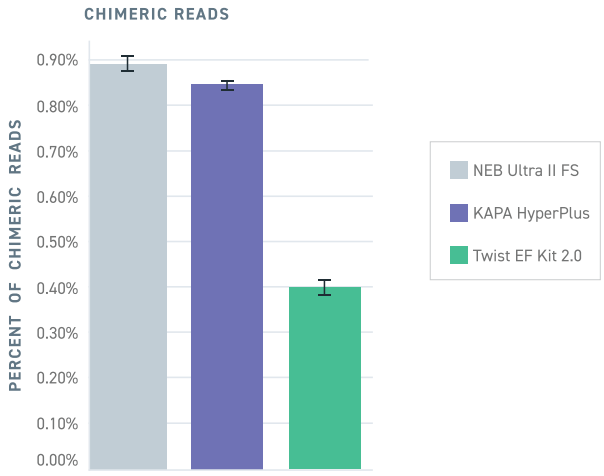


Figure 6. Error Rates of Amplification Master Mixes. Amplification master mixes Equinox and KAPA Hifi were used and misincorporation events were measured with a proprietary NGS-based assay. Values shown are the average error rate of all misincorporation events.

SEQUENCE ERROR RATE

Errors during amplification can be held to a minimum. The upgraded EF Kit 2.0 includes the Equinox Master Mix, featuring a high-fidelity, hot-start polymerase. Compared with a common industry amplification master mix, this mix has a lower error rate with fewer misincorporated bases (**Figure 6**). This provides users with more accurate amplification and higher-quality sequencing data.



CHIMERA RATE

Chimeras are sequencing reads that map to two different, non-overlapping locations on the target DNA. The presence of artificial chimeras generated during library preparation from inappropriate ligation or recombination during amplification steps can lead to sequencing errors and waste of sequencing space. The improved ligation module of EF Kit 2.0 paired with the new Equinox Master Mix significantly decreases the fraction of chimeric reads when compared with other commercially available kits (**Figure 7**).

Figure 7. Percent of Chimeric Reads in Whole Exome Sequencing. NGS libraries were prepared from 50 ng NA12878 human gDNA following protocol recommendations for a 200 bp insert library. Target enrichment was performed using Twist Target Enrichment Standard Hybridization v1 Protocol and the Twist Core Exome Panel. Sequencing was performed on an Illumina NextSeq 500/550 High Output v2 kit to generate 2x75 paired end reads. Data were downsampled to 150x raw coverage and the Picard metric PCT_CHIMERAS is reported. Error bars represent the standard deviation between replicates.

PERFORMANCE IN TARGET ENRICHMENT

Generating robust, high-quality libraries is the first step to superior target enrichment data. Twist's Library Preparation EF Kit 2.0 is part of an end-to-end target enrichment workflow that includes flexible options for panel size, plexity, and throughput. Whole exome libraries generated with EF Kit 2.0 and captured with our Twist Target Enrichment Standard Hybridization v1 Protocol show high library diversity and capture uniformity (lower fold-80 base penalty), while delivering equivalent, high on target rates (**Figure 8**).

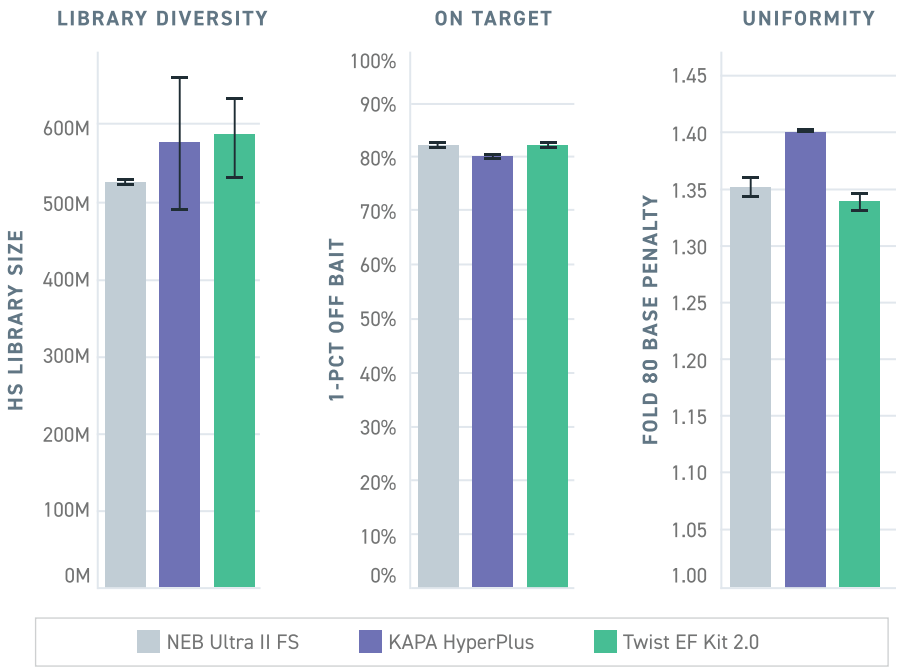


Figure 8. Key Capture Metrics. Libraries prepared from 50 ng NA12878 human gDNA following protocol recommendations for a 200 bp insert library were captured using Twist Target Enrichment Standard Hybridization v1 Protocol and the Twist Core Exome. Sequencing was performed on an Illumina NextSeq 500/550 High Output v2 kit to generate 2x75 paired end reads. Data was downsampled to 150x aligned coverage relative to the panel target size and analyzed using Picard Metrics.

CONCLUSION

The Twist Library Preparation EF Kit 2.0 includes all the reagents required for fragmentation, end-repair, dA-tailing, adapter ligation, and library amplification. It incorporates the enzymes for fragmentation of gDNA samples, allows for tunable insert sizes, and can be used over a broad range of DNA inputs. Improvements to the core reagents and inclusion of Equinox Amplification Mix produce final libraries with high fidelity and low chimeric read rates. This kit is highly customizable for use across a wide range of applications and seamlessly fits into Twist target enrichment workflows.

Read more about how Twist Library Prep EF Kit 2.0 can improve sequencing quality with low-error NGS library preparation here.

REAGENTS SUPPLY AND SUPPORT

Library Preparation Enzymatic Fragmentation Kit 2.0

104206	Twist Library Preparation EF Kit 2.0, 16 Samples
104207	Twist Library Preparation EF Kit 2.0, 96 Samples

Twist Universal Adapter System

101307	Twist Universal Adapter System TruSeq Compatible, 16 Samples
101308	Twist Universal Adapter System TruSeq Compatible, 96 Samples Plate A
101309	Twist Universal Adapter System TruSeq Compatible, 96 Samples Plate B
101310	Twist Universal Adapter System TruSeq Compatible, 96 Samples Plate C
101311	Twist Universal Adapter System TruSeq Compatible, 96 Samples Plate D

Twist UMI Adapter System

105040	Twist UMI Adapter System TruSeq Compatible, 16 Samples
105041	Twist UMI Adapter System TruSeq Compatible, 96 Samples Plate A
105042	Twist UMI Adapter System TruSeq Compatible, 96 Samples Plate B
105043	Twist UMI Adapter System TruSeq Compatible, 96 Samples Plate C
105044	Twist UMI Adapter System TruSeq Compatible, 96 Samples Plate D
105094	Twist UMI Adapters - TruSeq Compatible, 96 Samples

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