Twist FlexPrep UHT Library Preparation Kit

Ultra-high throughput with Normalization by Ligation

Despite the steady decline in sequencing costs, array-based technologies are still the tool of choice for many large-scale genomic studies. Traditionally, microarrays have offered affordability, a simple workflow, high-throughput capabilities, and high-confidence single nucleotide polymorphism (SNP) calling, areas where next-generation sequencing (NGS) can fall short. However, as researchers seek to extend genomic profiling beyond just SNPs, a truly high-throughput, cost-effective NGS solution is needed.

Twist's FlexPrep UHT Library Preparation Kit offers an NGS alternative to array-based technologies for population studies, agrigenomics, and other ultra-high throughput applications.

By combining Twist's Normalization by Ligation (NBL) technology with gold-standard enzymatic fragmentation methods, the Twist FlexPrep UHT Library Preparation Kit offers built-in sample normalization across a wide range of DNA inputs, reducing both cost and complexity of typical sample processing steps prior to library preparation. Early sample barcoding also enables downstream pooling of 12 separate samples into one reaction for a more streamlined and efficient workflow. Whether the research aim is imputing genomic variation across a whole genome or directly enriching regions of interest, this workflow removes typical throughput challenges facing NGS to enable studies at any scale.

KEY BENEFITS

Normalization by Ligation (NBL)

- Eliminate upfront normalization
- No need for intermediate quantitation
- Work with sample batches using a wide range of inputs (30 ng to 300 ng)

Early Sample Pooling Saves Cost

- Pool 96 samples into 8 tubes after the ligation reaction
- Miniaturized reaction volumes upstream
- Reduction in consumables downstream

Designed for Ultra High-Capacity Multiplexing

- Uniquely index up to 1,152 samples from a single kit
- Increase capacity 12x with existing lab setup*
- Supports 96 samples per target enrichment reaction

Eliminate upfront normalization and intermediate quantitation of samples

The Twist FlexPrep UHT Library Preparation Kit takes a novel Normalization by Ligation approach which eliminates the need for upfront and intermediate sample quantitation, streamlining your sequencing workflow (**Figure 1**).

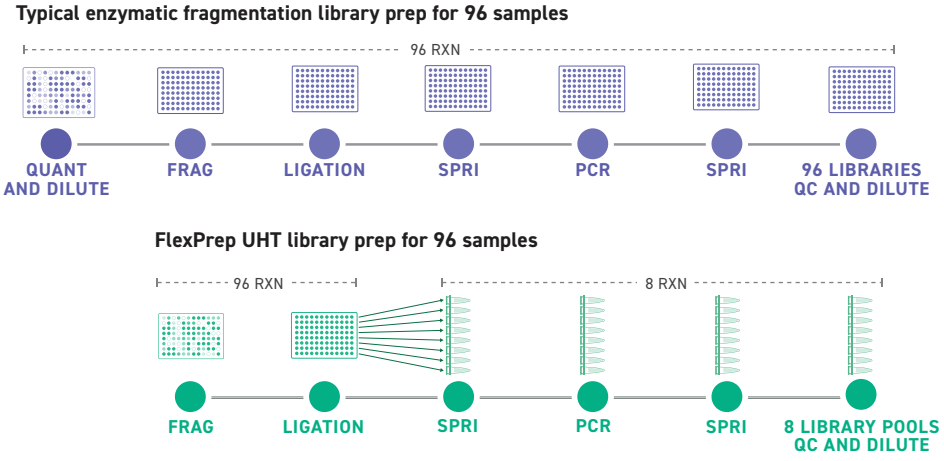


Figure 1. Comparison of typical enzymatic fragmentation (EF) to Twist FlexPrep UHT Library Preparation Kit using 96 samples. Typical library preparation requires labor-intensive quantitation and dilution prior to fragmentation. Reaction footprint also scales linearly with the number of samples. The Twist FlexPrep UHT Library Preparation Kit self-normalizes during ligation, and leverages inline barcoded adapters to enable a high-throughput workflow by compressing every 12 reactions into one pooled reaction.

For Research Use Only. Not for use in diagnostic procedures.

Purpose-built workflow to save resources without compromising quality

Twelve inline barcodes are included on the FlexPrep normalization adapters, which allow for an ultra-high throughput and automation-friendly multiplexing strategy (**Figure 2**).

With the FlexPrep UHT Library Preparation Kit, sequencing throughput can be maximized by running up to 1,152 samples in a single sequencing run from one kit. This increased efficiency can translate to cost and consumables savings.

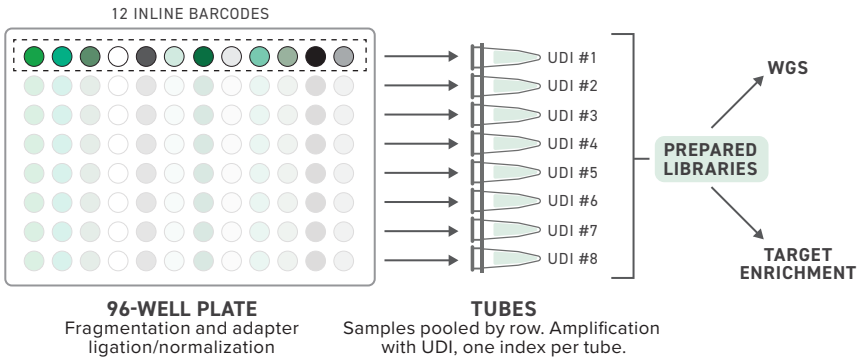
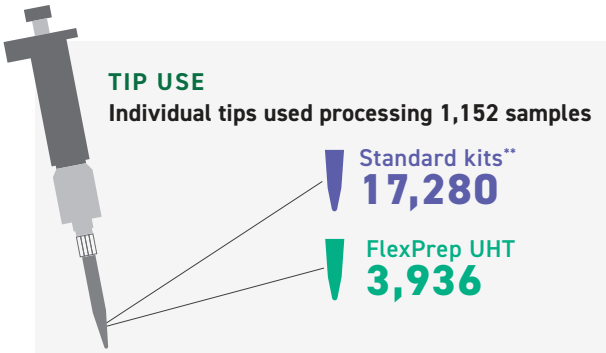


Figure 2. Simplicity of FlexPrep workflow post-ligation. Fragmentation and ligation reactions are prepared in one well per sample. The adapters used during ligation contain inline barcodes that allow for the pooling of all 12 wells in a row of a 96-well plate. Individual pools are prepared with indices (UDIs) added by PCR for pool-level demultiplexing, supporting a less complex library preparation process post-ligation.



TIP USE

Individual tips used processing 1,152 samples

Standard kits** **17,280**

FlexPrep UHT **3,936**

REAGENT WASTE SAVINGS

Designed to decrease the amount of

- Ethanol used for clean up (12 x less)
- AMP mix for post-ligation (12 x less)
- SPRI beads and ethanol post-AMP (12 x less)

FlexPrep normalizes yield while maintaining constant insert sizes

The self-normalizing adapters found in the Twist FlexPrep UHT Library Preparation Kit supports mass inputs of 30 ng to 300 ng with minimal impact on uniformity and on insert size for whole genome sequencing (WGS) or target enrichment (TE) workflows (**Figures 3 and 4**).

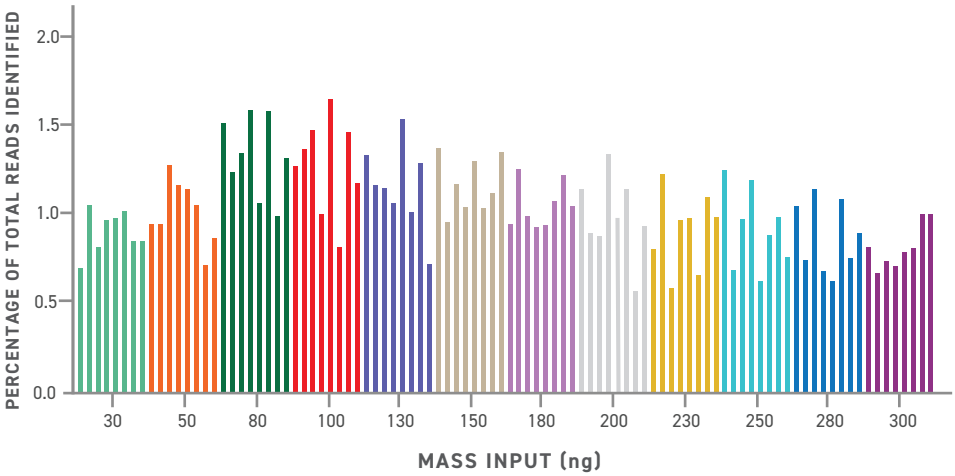


Figure 3. NGS read depth normalization with variable DNA mass input. 96 libraries were prepared with human genomic DNA (gDNA) (NA12878) using the Twist FlexPrep UHT Library Preparation Kit and FlexPrep Target Enrichment Protocol as a 96-plex with a custom 800 kb panel before sequencing on an Illumina NextSeq 550. Eight library pools of 12 samples each were generated using variable mass inputs ranging between 30 ng to 300 ng in each library pool. Percentage of total read counts identified to each library is calculated after unique dual index and inline barcode demultiplexing. Average with perfect normalization is 1.04% (100/96).***

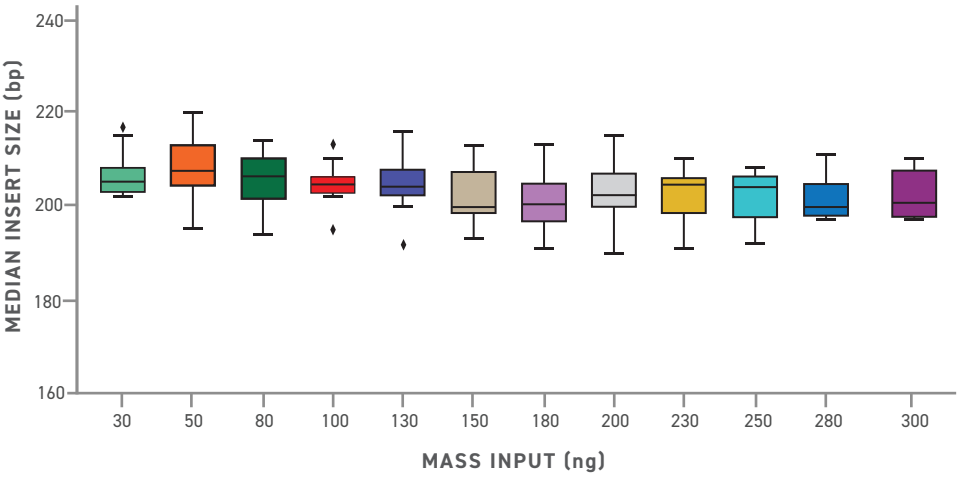


Figure 4. Median insert size with variable DNA mass input. A 96-plex capture using the Twist FlexPrep Target Enrichment Protocol across a 10-fold difference in gDNA mass input and sequenced on an Illumina NextSeq 550 shows consistent median insert sizes of 210 bp to 220 bp. Data were downsampled to 75X of target size and Picard metric MEDIAN_INSERT_SIZE was reported.

High multiplexing uniformity with less over-sequencing

While CV can be used to define normalization on the basis of comparing the most and least sequenced samples, using the least sequenced sample by itself is a more accurate measurement of normalization. With increasing sequencing coverage (over-sequencing) a given pool will have a higher proportion of samples reach the desired coverage (**Figure 5**). Minimizing the required over-sequencing to maximize the proportion of libraries in a pool that reach the desired coverage is the goal of normalization protocols.

We created the over-sequencing (S) metric, which measures how much relative sequencing is needed to catch up the least sequenced sample with the mean sequencing depth of the pool. A lower value indicates less variability among the coverage of the individual samples; a hypothetical case of perfect uniformity would have an S metric of 1. Using the FlexPrep UHT Library Prep kit with pools of constant or various DNA mass inputs require minimal >1.5 for constant and >1.75 for various over-sequencing (**Figure 6**).

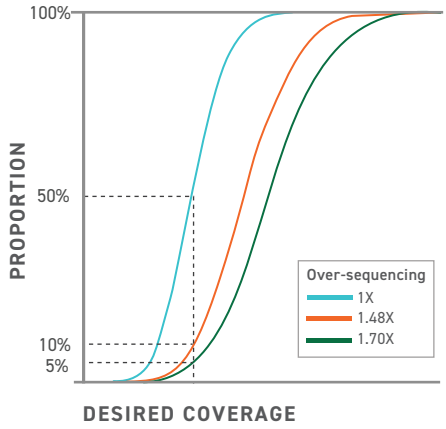


Figure 5. Fold over-sequencing necessary to raise % of samples in "non-zero-samples" to desired mean coverage. Graph of proportion of libraries in a pool that have a desired sequencing coverage with increasing over-sequencing. A 96-plex FlexPrep workflow, with 48% and 70% extra sequencing coverage needed to bring 90% and 95% of samples to desired mean coverage respectively.

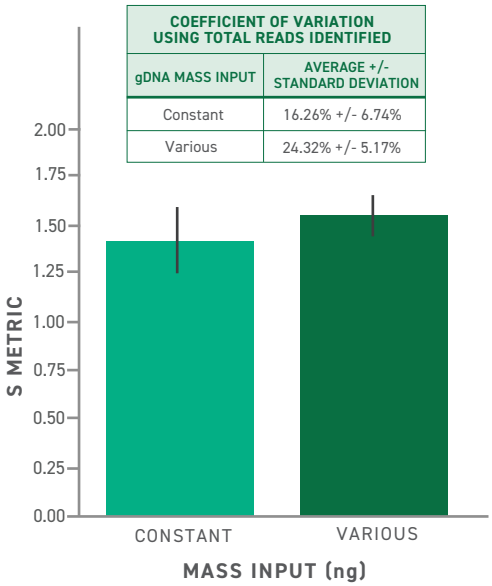


Figure 6. S metric of final FlexPrep library pools. When using the S metric to compare library pools generated using the Twist FlexPrep UHT Library Preparation Kit, we show normalization using gDNA mass input at a constant and variable mass ranging between 30 ng and 300 ng. Table with respective CVs added above for comparison.

Identify your regions of interest with target enrichment

FlexPrep's workflow efficiencies also extend to target enrichment where a single enrichment reaction can be used for up to 96 samples.

Like other Twist library preparation kits, FlexPrep UHT generates high-complexity libraries that have uniform coverage after target enrichment (**Table 1**). In combination with Twist Custom Panels, FlexPrep offers tunable coverage of SNPs, k-mers, structural variants, and other genomic features with the potential to interrogate millions of markers.

TARGET ENRICHMENT USING A 96-PLEX 30 NG TO 300 NG gDNA MASS INPUT INTO LIBRARY PREPARATION	
METRICS AT 75X RAW TARGET COVERAGE	AVERAGE +/- STANDARD DEVIATION
Selected Bases	79.22% +/- 0.54%
Mean Target Coverage	32.74 +/- 4.78
Chimeras	1.31% +/- 0.38%
Fold-80 Base Penalty	1.416 +/- 0.044
Covered Bases at 10X	96.06% +/- 0.94%
Covered Bases at 20X	85.41% +/- 6.36%
Covered Bases at 0X	0.43% +/- 0.04%

Table 1. Metrics from a 96-plex enrichment using the Twist FlexPrep UHT Library Preparation Kit and FlexPrep Target Enrichment Protocol. A single 96-plex target enrichment was performed with an 800 kb panel with gDNA mass input ranging between 30 ng to 300 ng into library generation. The enriched material was sequenced on an Illumina NextSeq 550 and downsampled to an average of 75x coverage. Key target enrichment metrics from Picard are reported above.

**Based on an 8-well vs a 96-well workflow*

***Standard Kits based on workflows which use enzymatic fragmentation followed by ligation and PCR*

****For all charts, figures, and graphs per Twist internal data September 2024.*

LEARN MORE

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ORDERING INFORMATION

192 Sample Kits
109220: Twist FlexPrep UHT Library Preparation Kit, 192 Samples
109223: Twist FlexPrep UHT LP and Hybridization Kit, 192 Samples

1,152 Sample Kits
109224: Twist FlexPrep UHT Library Preparation Kit, 1152 Samples
109226: Twist FlexPrep UHT LP and Hybridization Kit, 1152 Samples

Supplementary kits for higher-throughput applications
116207: Twist FlexPrep UHT LP Kit, 1152 Samples, Plate A
116217: Twist FlexPrep UHT LP and Hyb Kit, 1152 Samples, Plate A
116218: Twist FlexPrep UHT LP Kit, 1152 Samples, Plate C
116220: Twist FlexPrep UHT LP and Hyb Kit, 1152 Samples, Plate C
116221: Twist FlexPrep UHT LP Kit, 1152 Samples, Plate D
116222: Twist FlexPrep UHT LP and Hyb Kit, 1152 Samples, Plate D