Twist cfDNA Library Preparation Kit

KEY BENEFITS

Robust Performance with Streamlined Workflow

- High conversion rate reliably detects rare variants (≤0.1% VAF)
- Produces high coverage libraries compatible with single-UMI or duplex-UMI workflows
- Reliable and robust performance with a sample input range of 1–20 ng
- Sequence-ready libraries in 2 hours

The Twist cfDNA Library Preparation Kit addresses challenges associated with library preparation from circulating cell-free DNA (cfDNA). Liquid biopsies have gained prominence in oncology research, particularly for investigating potential biomarkers within genetic material from tumors circulating in peripheral blood. This kit enables high conversion cfDNA library generation for next-generation sequencing (NGS) on Illumina systems, overcoming the technical challenges associated with low-input and degradation of cfDNA, as well as other samples that are hard to acquire and have limited DNA abundance (i.e. urine and CSF samples).

The Twist cfDNA Library Preparation Kits provide our highest quality library preparation solution, offering not only robust performance with low sample input, but also very high conversion rates.

Figure 1: The Twist cfDNA Library Preparation Kit and Twist cfDNA Library Preparation and Hyb Mix Kit enable WGS as well as targeted enrichment workflows

Efficiency redefined

Revolutionize your liquid biopsy research with the streamlined and high-performance Twist cfDNA Library Preparation protocol, designed to deliver results with high efficiency in 2 hours. This protocol minimizes sample handling, ensuring a simplified workflow for researchers in the field. The process can be done in three steps:

1. End Repair and dA-Tailing: A single step End Repair and dA-Tailing without the need to perform clean-up.
2. Ligation: Rapid 15-minute incubation time to integrate UMI adapters with high conversion rates and low artifacts.
3. PCR Amplification: Amplification and indexing for sample multiplexing.

With an emphasis on efficiency, precision, and ease of use, the Twist cfDNA Library Preparation protocol stands as a testament to our commitment to advancing liquid biopsy research methodologies, providing researchers with a powerful tool to unlock the full potential of cfDNA analysis.

Enhanced workflow for superior conversion in cfDNA samples

The Twist cfDNA Library Preparation Kit, combined with an updated Target Enrichment Workflow, ensures exceptional conversion of input DNA molecules into sequence-ready libraries. In liquid biopsy research, where increased complexity translates to higher sensitivity of detection, this improved conversion rate is critical. In comparison to other NGS library preparation kits recommended for cfDNA (**Figure 2**), our approach leads to increased library yield and ultimately increased complexity.

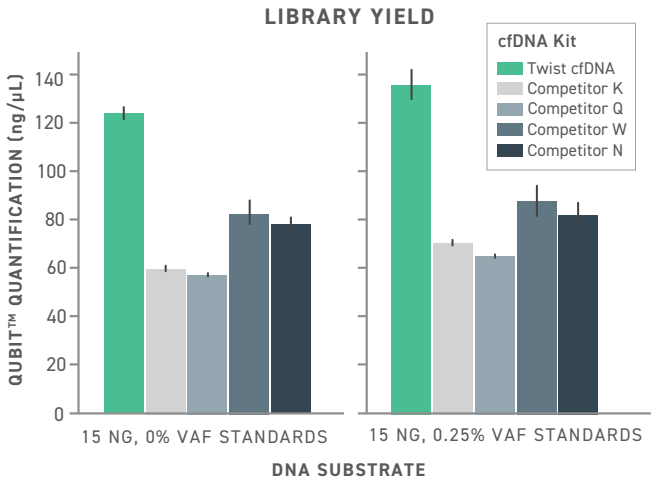

Figure 2: Twist cfDNA Library Preparation Kit delivers higher cfDNA library yields. Following library generation, samples were eluted in 20 µL of water and 1 µL was quantified with Qubit™ dsDNA Broad Range Kit.

This improved conversion measured by Qubit™ translates to high values for mean target coverage. When combined with unique molecular identifiers (UMIs) for deduplication and bioinformatic error correction, we can observe consistently deep coverage (**Figure 3**). Increasing unique coverage improves the likelihood to reliably detect low-frequency variants.

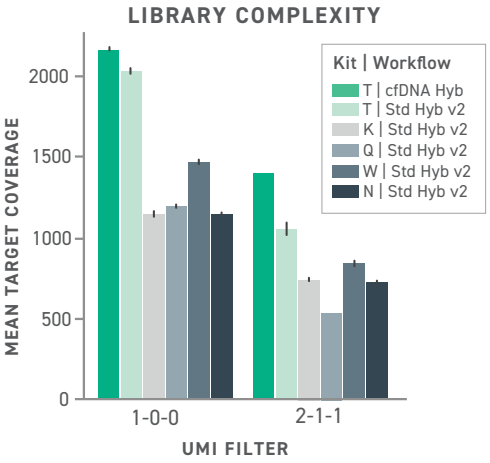

Figure 3: Twist cfDNA Library Preparation and Hyb Mix Kit delivers higher complexity with and without UMI deduplication. Libraries were single-plex captured with a custom 50kb oncology panel targeting variant sites in the cfDNA standard material with present or updated TE recommendation.

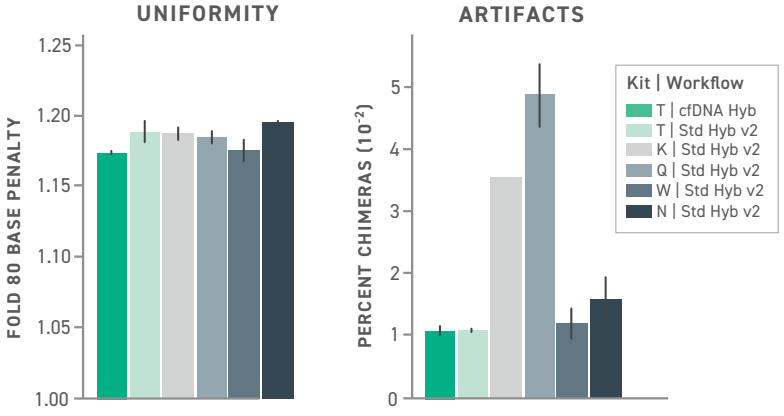

Figure 4: Twist cfDNA Library Prep and Hyb Mix Kit yields high uniformity with minimal artifacts. Picard hybrid-selection and alignment metrics are reported following UMI collapse with 1-0-0 fgbio UMI filtering parameters. Lower Fold 80 Base Penalty is preferred as it corresponds to better uniformity across target sites, and lower Percent Chimeras is preferred to minimize artifacts.

Improved coverage with UMI benefits low VAF detection

The Twist cfDNA Library Preparation Kit converts more of your cfDNA input into sequenceable libraries, resulting in more variants detected at lower variant allele frequency (VAF) when compared to competitors.

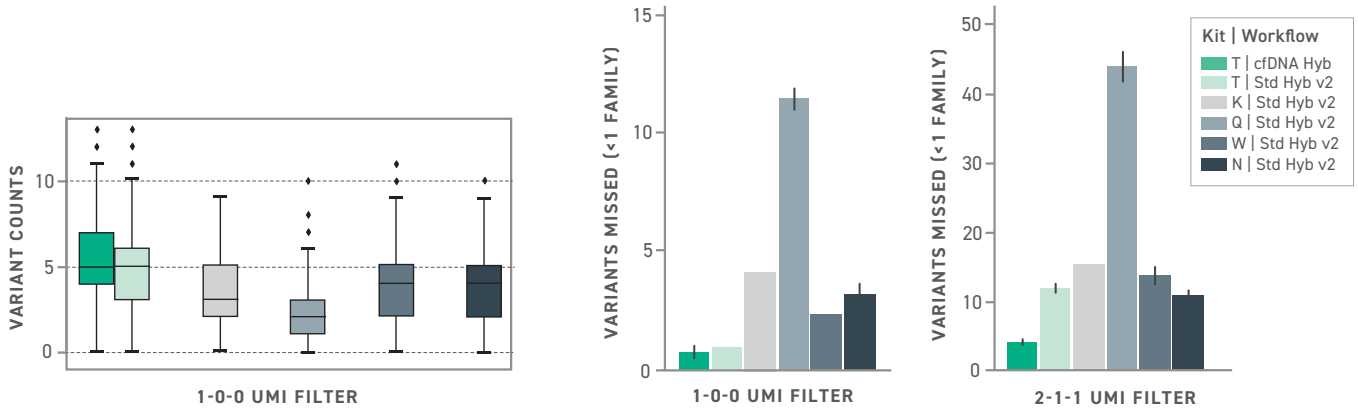

Figure 5: Twist cfDNA Library Preparation and Hyb Mix Kit enables sensitive and accurate detection of variants at 0.25% VAF. Libraries were single-plex captured with a custom 50kb oncology panel targeting 217 SNP variant sites in the Pan-Cancer standard material. Variants Missed is calculated by summing sites with less than 1 variant family.

Sample compatibility with native cfDNA type

The high library conversions observed with control samples were also replicated using native cfDNA libraries. In addition, an ultra low-input of 1ng, was included demonstrating the quality of conversion against a range of low-input masses. These results strongly demonstrate that the Twist cfDNA library preparation workflow yields excellent conversion and variant detection on real samples which is consistent with the above control measurements.

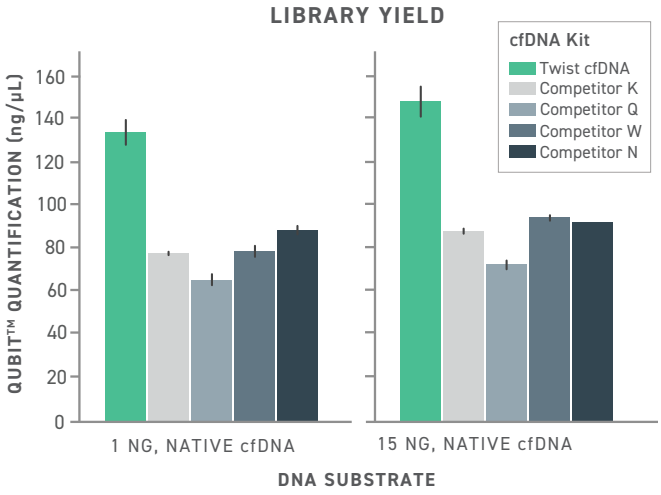

Figure 6: Twist cfDNA Library Preparation Kit delivers higher cfDNA library yields with native cfDNA samples. Following library generation, samples were eluted in 20 µL of water and 1 µL was quantified with Qubit™ dsDNA Broad Range Kit.

Optimal data fidelity in cfDNA samples

Twist cfDNA Library Preparation Kit provides high-conversion of cfDNA into sequenceable molecules without compromising sample quality and integrity. High-conversion of molecules will result in a uniform sequencing coverage and low chimeric artifacts (**Figure 9**).

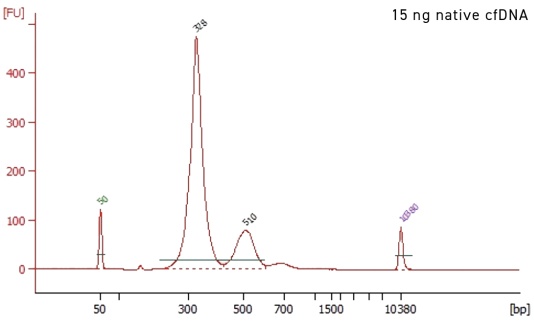

Figure 7: Twist cfDNA Library Preparation Kit is compatible with native cfDNA samples. Libraries were made with 15 ng of native cfDNA with 8-cycles of PCR. 1 µL of the final library was loaded onto an Agilent Bioanalyzer 7500 chip.

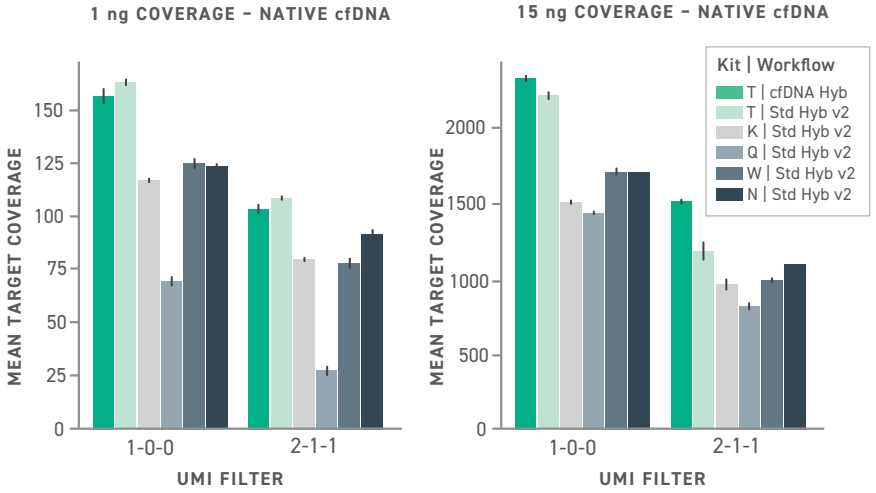

Figure 8: Twist cfDNA Library Preparation and Hyb Mix Kit is compatible with native cfDNA samples. Libraries were single-plex captured with a custom 50kb oncology panel targeting variant sites in the standard material.

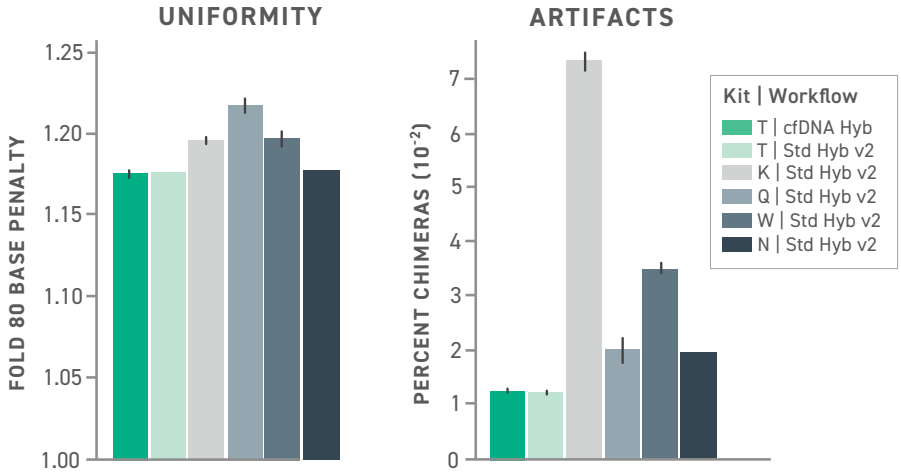

Figure 9: Twist cfDNA Library Preparation and Hyb Mix Kit yields high uniformity with minimal artifacts with 15 ng of native cfDNA. Picard hybrid-selection and alignment metrics are reported following UMI collapse with 1-0-0 fgbio UMI filtering parameters. Lower Fold 80 Base Penalty is preferred as it corresponds to better uniformity across target sites, and lower Percent Chimeras is preferred to minimize artifacts.

Methods

The data presented here was generated from libraries created with the Twist cfDNA Library Preparation Kit and competitor kits K, Q, W, and N. For each kit we tested four different starting substrates: 15 ng of Twist cfDNA Pan-Cancer Reference Standards v2 0.25% VAF, 15 ng of Twist cfDNA Pan-Cancer Reference Standards v2 0% VAF, 1 ng of native cfDNA (Plasmalab), and 15 ng of native cfDNA (Plasmalab). After ligation, indexing PCR cycles were standardized across all kits. 1 ng input samples were amplified 12 PCR-cycles while 15 ng input samples were amplified 8 PCR-cycles. Final elution was into 20 µl water.

After library preparation, all libraries were hybridized according to the Twist Target Enrichment Standard Hybridization v2 protocol with 500 ng input into capture. This hybridization workflow is referred to as “Std Hyb v2” in the data. In addition, Twist libraries were also hybridized according to the updated cfDNA Target Enrichment Standard Hybridization protocol released alongside this kit with 80 ng and 1200 ng input into capture for 1 ng samples and 15 ng samples respectively. This hybridization workflow is referred to as “cfDNA Hyb” in the data. We included this distinction to show the compounding improvement of the Twist cfDNA Library Preparation Kit and the updated hybridization workflow over competitors. Captured libraries were pooled and sequenced paired end with 80,000x coverage on a Nextseq550 150-cycle kit (Illumina). UMI deduplication was performed with fgbio using 1-0-0 or 2-1-1 UMI flag filters and metrics are computed from Picard.

LEARN MORE

twistbioscience.com/ngs

sales@twistbioscience.com

ORDERING INFORMATION

cfDNA Library Prep
107603: Twist cfDNA Library Preparation Kit–16 Samples
107604: Twist cfDNA Library Preparation Kit- 96 Samples

cfDNA Library Prep and Hybridization
107609: Twist cfDNA Library Preparation and Hyb Mix Kit–16 Samples and 2 Reactions
107610: Twist cfDNA Library Preparation and Hyb Mix Kit–96 Samples and 12 Reactions