Twist Bioscience Library Preparation and Target Enrichment on the Biomek i7 Hybrid

Introduction

The Twist Bioscience Library Preparation and Target Enrichment assay is a highly modular target enrichment next generation sequencing (NGS) kit with a variety of applications from fixed panels to whole exome sequencing. The kit utilizes fragmentation, ligation, and amplification of genomic DNA (gDNA) to prepare NGS libraries and bead-based capture of hybridized libraries to enrich libraries. Twist Bioscience offers users a high degree of flexibility to suit the needs of the laboratory, including either enzymatic or mechanical fragmentation, two different sets of indexing chemistries using Twist's full length Combinatorial Dual (CD) Index Adapters or Universal Dual-Indexed (UDI) Primers, single or multi-plex enrichment options, a selection of commercially available fixed panels and custom panel options for library enrichment, and a "standard" 16 hour hybridization option or a "fast" hybridization option that can run for 15 minutes to 4 hours. The entire manual library preparation and targeted enrichment protocols can be completed in as little as one day or up to three days.

In this application note, we describe and demonstrate the automated performance of the Twist Enzymatic Fragmentation (EF) library preparation with UDI Primers and both the Twist Target Enrichment and Fast Hybridization Target Enrichment workflows using the Twist Human Core Exome panel on the Biomek i7 Hybrid Automated Workstation. The automation solution can support library construction from 1 to 96 samples and target capture of between 1 and 48 library pools, allowing the user any plexity between 1 and 8. The automated protocol supports the use of both EF and mechanical fragmentation (MF) of gDNA and use of either CD or UDI adapters for library preparation and both a standard 16-hour hybridization or a fast 15 minute to 4 hour hybridization for library enrichment.

In comparison to the manual pipetting, the Twist Bioscience Library Preparation and Target Enrichment workflows on the Biomek platform provides:

- Reduced hands-on time and increased throughput
- Reduction in potential pipetting errors
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Knowledgeable support

Spotlight

Biomek i7 Hybrid (Multichannel 96, Span-8) System features deliver reliability and efficiency to increase user confidence and walk-away time

- 300 µL or 1200 µL Multichannel head with 1-300 µL and 1-1200 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with 45 positions
- Orbital Shakers, peltiers Span-8 and 96 channel Tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)

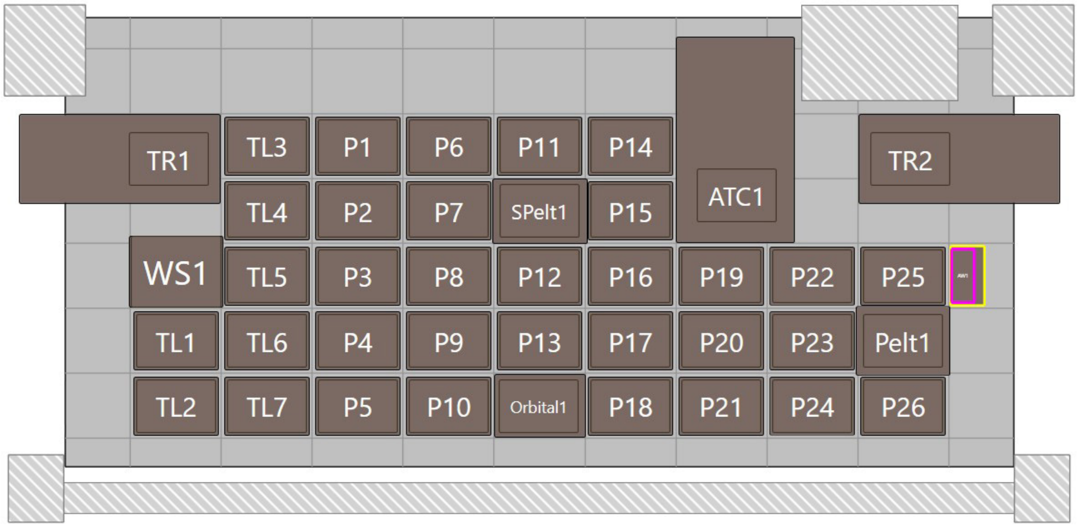


Figure 1. Biomek i7 Automated Workstation Deck Layout. The layout shown above was used in the demonstration of the Twist Bioscience Library Preparation and Target Enrichment automated method.

Automated Method

The automated Twist Library Preparation and Enrichment method is constructed to reflect the modularity of Twist's NGS chemistry, both following the assay manufacturer's recommended start and stop points and allowing the user to select the parameters that match the chemistry they are running. This modularity allows for the method to be deployed in pre- and post-PCR laboratories. With the exceptions of mechanical shearing, library quality check and library pool dry down via vacuum concentrator, all steps of the automated method can be performed on-deck. Target enrichment requires the use of the on-deck thermocycler to perform hybridization, heat wash buffers, and incubate capture washes of the library pools.

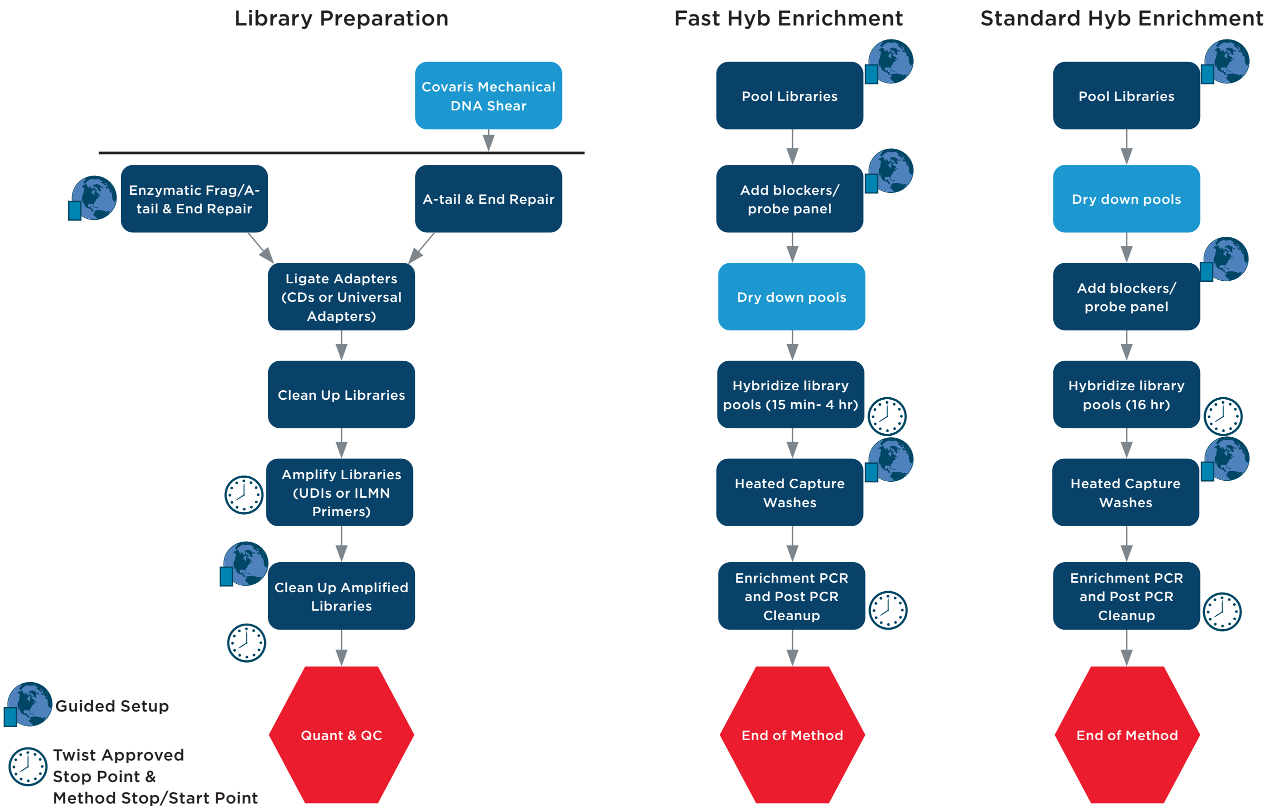


Figure 2. Twist Library Preparation and Target Enrichment automated method workflow. Steps in light blue indicate manual processes in the workflow. Steps in dark blue indicate steps that are automated on the Biomek i7 Hybrid. Red hexagons indicate the end of a given workflow or required quantification or sample quality control assays.

Enzymatic Fragmentation/UDI Adapter Library Prep	24 Libraries	96 Libraries
Prepare Reagents/Set Up Biomek	30 min	45 min
Method Run	1 hr, 44 min	2 hr, 13 min
Total	2 hr, 14 min	2 hr, 58 min

Standard Hyb Enrichment	8 Pools	48 Pools
Prepare Reagents/Set Up Biomek	30 min	45 min
Pooled Normalized Library Drydown	1 hr	1 hr
Method Run	19 hr, 6 min	19 hr, 32 min
Total	20 hr, 36 min	21 hr, 17 min

Fast Hyb Enrichment	8 Pools	48 Pools
Prepare Reagents/Set Up Biomek	30 min	45 min
Pooled Normalized Library Drydown	1 hr	1 hr
Method Run	4 hr, 50 min	5 hr, 36 min
Total	6 hr, 20 min	7 hr, 21 min

Table 1. Estimated run times for Twist Library Preparation and Target Enrichment automated method on the Biomek i7 Hybrid Automated Workstation. Timing estimates for method run times were obtained from Biomek software. Timing estimates include incubations and thermocycling, but do not include sample preparation or reagent thawing. Hybridization incubation time for standard hybridization setup was estimated at 16 hours to simulate an overnight hybridization. Hybridization incubation time for fast hybridization setup was estimated at 2 hours.

The software provides several user-friendly features such as

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument if needed. The automated method may also be run through the standard Biomek software if Biomek Method Launcher is unavailable.

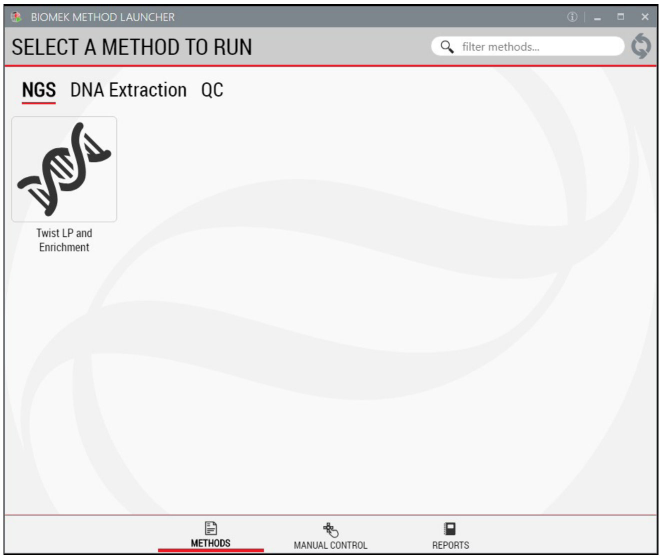
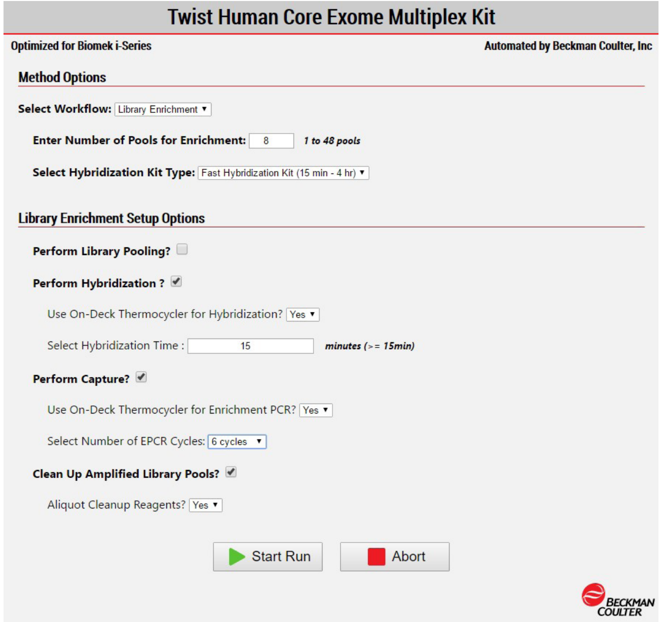


Figure 3. Biomek Method Launcher provides an easy interface to launch the method.

2. Method Options Selector (MOS)

MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability, and the ease of method execution.



Twist Human Core Exome Multiplex Kit

Optimized for Biomek i-Series

Automated by Beckman Coulter, Inc

Method Options

Select Workflow: Library Enrichment

Enter Number of Pools for Enrichment: 8 *1 to 48 pools*

Select Hybridization Kit Type: Fast Hybridization Kit (15 min - 4 hr)

Library Enrichment Setup Options

Perform Library Pooling? ☐

Perform Hybridization ? ☑

Use On-Deck Thermocycler for Hybridization? Yes

Select Hybridization Time : 15 *minutes (>= 15min)*

Perform Capture? ☑

Use On-Deck Thermocycler for Enrichment PCR? Yes

Select Number of EPCR Cycles: 6 cycles

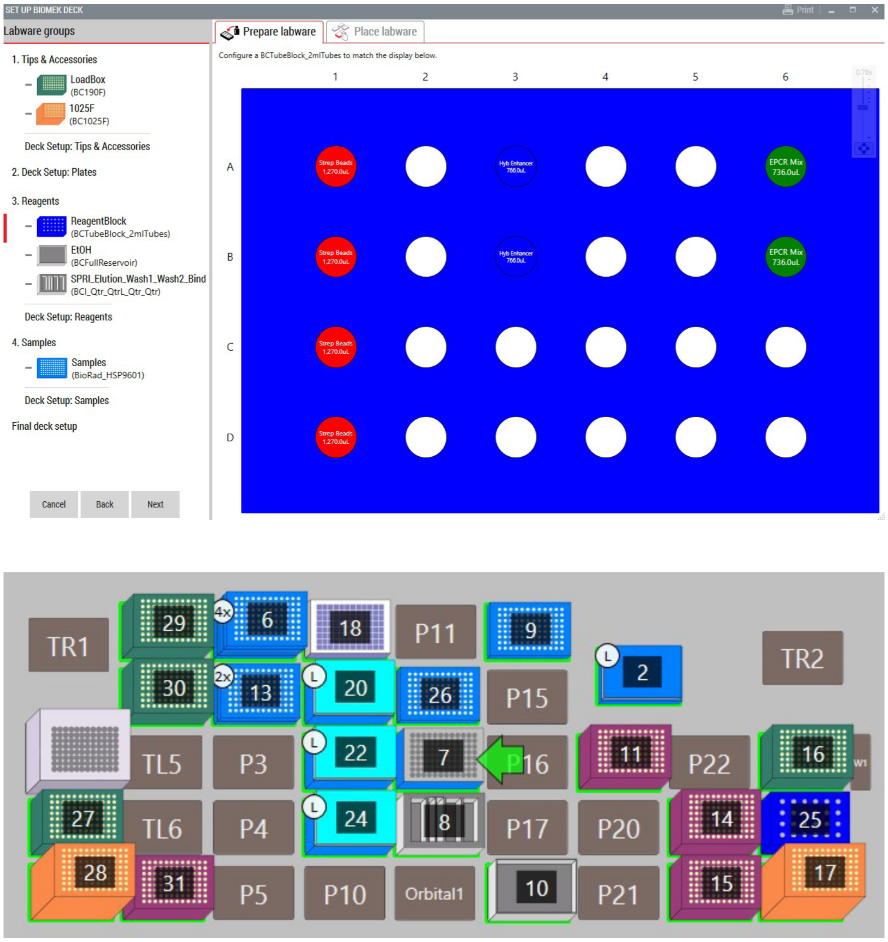
Clean Up Amplified Library Pools? ☑

Aliquot Cleanup Reagents? Yes

Figure 4. Biomek Method Option Selector enables users to select the desired workflow, sample number, and a variety of workflow customization options.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS and provides the user specific graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents. DeckOptix Final Check (part of BML) utilizes the Biomek's built-in camera system to verify labware placement to provide another layer of protection against incorrect instrument setup.



Configure a BCTubeBlock_2mlTubes to match the display below.

	1	2	3	4	5	6
A	Strep Beads 1,270.0uL		Hyb Enhancer 766.0uL			EPCR Mix 736.0uL
B	Strep Beads 1,270.0uL		Hyb Enhancer 766.0uL			EPCR Mix 736.0uL
C	Strep Beads 1,270.0uL					
D	Strep Beads 1,270.0uL					

Figure 5. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

Materials and Methods

The Biomek i7 Hybrid Automated Workstation was used in all steps of enriched library preparation unless otherwise noted. NGS libraries were prepared using 50 ng of Tru-Q 0 NGS DNA Multiplex DNA Reference Standards (Horizon) and Twist Enzymatic Fragmentation and Universal Adapter System kit. Prepared libraries were manually quantified using the Qubit™ dsDNA Broad Range Quantitation Assay (Thermo Fisher) and library size was determined using the Bioanalyzer High Sensitivity DNA kit (Agilent). Libraries were normalized to 187.5 ng and pooled to an 8-plex. Normalized, pooled libraries were combined with the Twist Core Exome Panel and Twist Universal Blockers and dried down off-deck using a Vacufuge™ (Eppendorf™) set to room temperature. Dried down pools were loaded onto the Biomek i7 Hybrid Automated Workstation for the remainder of the protocol. Pools were hybridized for 2 hours and subsequent enrichment was performed using the Twist Fast Hybridization and Wash Kit. Additionally, manually prepared libraries from Twist were enriched using the Twist Standard Hybridization and Wash Kit on the Biomek i7 Hybrid as per user manual specifications.

Final library QC was performed using the Qubit™ 1x dsDNA High Sensitivity Assay (Thermo Fisher) and Bioanalyzer High Sensitivity DNA kit. 16 technical replicates from two 8-plex pools for both enrichment kits were sequenced on an Illumina NextSeq using a 2x74 bp Paired End run using High Output v2.5 chemistry. Sequencing data was downsampled to 150x coverage and analyzed by Twist Bioscience using Picard Tools (Broad Institute).

Results

Following sequencing, final library insert size was examined to confirm insert size meets Twist Bioscience's expectations (Figure 6). The sixteen replicates were plotted for insert size in bp vs insert size count and were used to calculate average insert size. Per Twist's specifications, the target mean insert size should be at least 180 bp. Mean insert size for the Twist Standard Hybridization and Wash Kit was 183 bp and mean insert size for the Twist Fast Hybridization and Wash Kit was 188 bp.

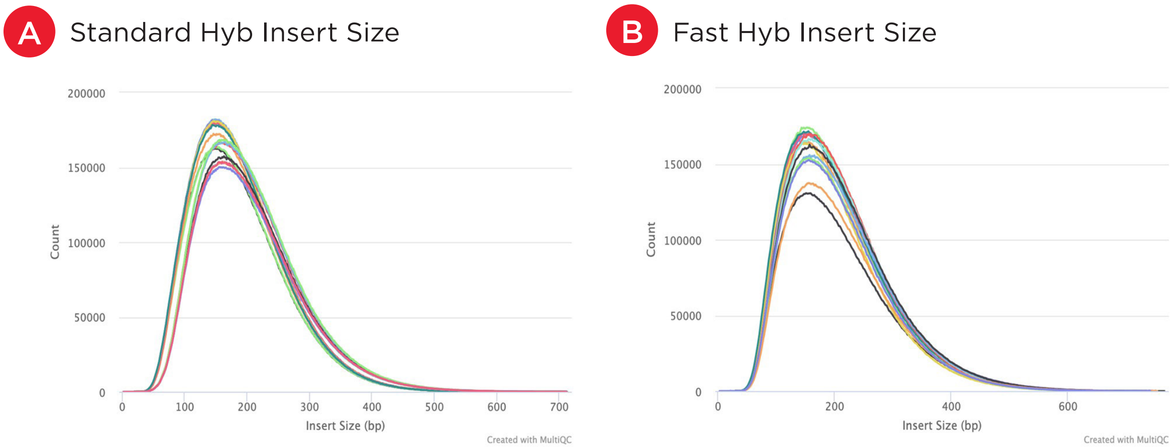


Figure 6. Insert sizes of libraries enriched with Twist hybridization/capture chemistries. Traces were generated using the data generated from the Picard Tools using the MultiQC tool v1.9 (SciLifeLab) and display insert size along the x-axis vs. insert size count along the y-axis. **(A)** Insert size sequencing data distribution for 16 libraries generated from the Twist Target Enrichment Protocol. **(B)** Insert size sequencing data distribution for 16 libraries generated from the Twist Fast Hybridization Target Enrichment Protocol.

Percent on target refers to the percentage of sequencing reads that map back to the region of interest and is a metric used to determine the quality of an enrichment procedure (Figure 7). Per Twist specifications, on target percentage should be above 75%. Mean percent on target is 81.1% for libraries enriched with the Standard Hybridization and Wash kit and 85.9% for libraries enriched with the Fast Hybridization and Wash kit.

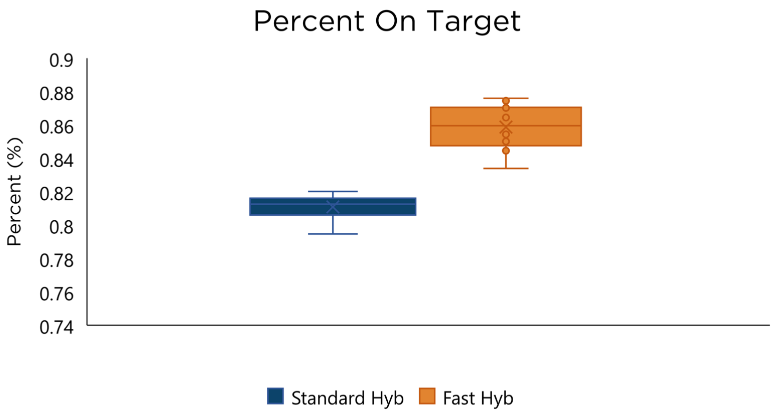


Figure 7. High percentage of on targets observed in libraries enriched with Standard and Fast Hybridization chemistries.

Fold-80 ratios describe the efficiency of the enrichment procedure and indicate the uniformity of enriched libraries. (Figure 8). Expected Fold-80 ratios should be below 1.5 to indicate high uniformity for libraries. Libraries prepared from the Twist Standard Hybridization and Wash Kit and the Twist Fast Hybridization and Wash Kit had a Fold-80 ratio of 1.34 and 1.41, respectively.

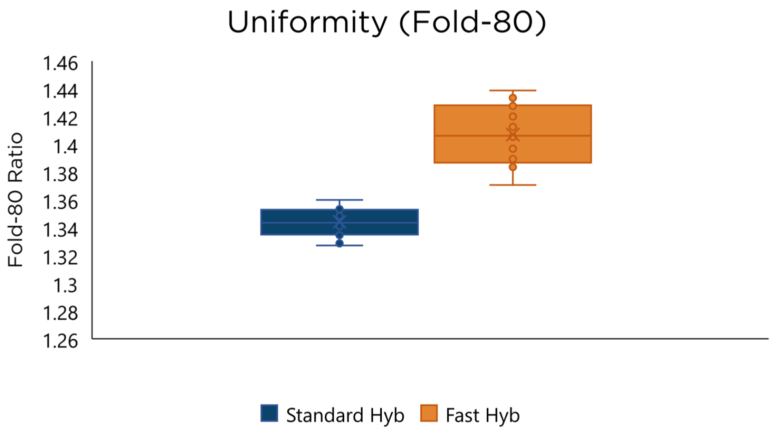


Figure 8. Fold-80 ratios for the Twist Standard and Fast Hybridization and Wash kits.

Sequencing coverage measures the depth of sequencing coverage across the targeted regions of the Twist Core Exome Panel (Figure 9). For each of the 16 technical replicates for both enrichment chemistries, over 90% of the targeted regions were covered to a depth of at least 30X, indicating an efficient hybridization and capture process.

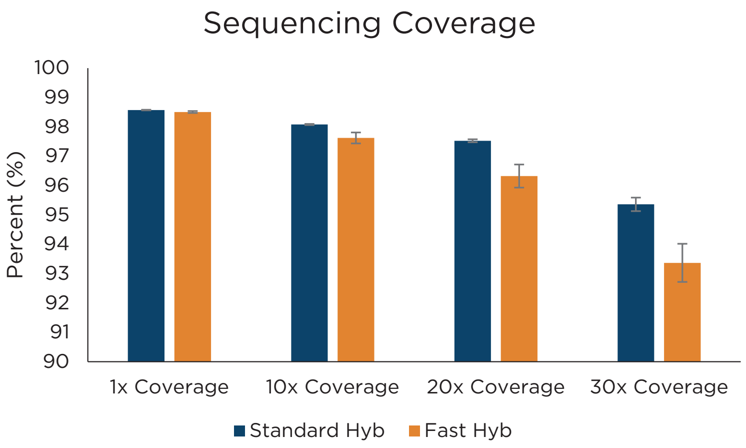


Figure 9. Target coverage of enriched libraries using Twist Standard and Fast Hybridization and Wash kits.

Conclusion

We have demonstrated a complete library preparation and enrichment workflow using Twist's NGS chemistries on the Biomek i7 Hybrid Automated Workstation with minimum user intervention. Prepared enriched libraries using either the Standard or Fast Hybridization and Wash kits result in high-quality, sequence-ready libraries that meet Twist Bioscience's quality metrics. The automated method by Beckman Coulter Life Sciences has been designed to work with any combinations of Twist's modular library preparation and enrichment chemistries.

Twist Bioscience Enzymatic Fragmentation and Universal Adapter System library preparation kit, Twist Bioscience Fast Hybridization and Wash kit, and Twist Bioscience Standard Hybridization and Wash kit are for Research Use Only and are not for use in diagnostic procedures. Beckman Coulter makes no warranties of any kind whatsoever express or implied, with respect to this protocol, including but not limited to warranties of fitness for a particular purpose or merchantability or that the protocol is non-infringing. All warranties are expressly disclaimed. Your use of the method is solely at your own risk, without recourse to Beckman Coulter. This protocol is for demonstration only, and is not validated by Beckman Coulter.

Biomek i-Series Automated Workstations are not labeled for IVD use and are not intended or validated for use in the diagnosis of disease or other conditions.

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