Automating Twist Bioscience's Modular Library Preparation and Targeted Enrichment Protocols Using Beckman Coulter Automation

Randy B. Pares, MS[1], Alex Sahm[2], Martin Giner, Ph.D.[1] Elena Tavkin, MS[1], Richard Gantt, Ph.D.[2], Tina Han, Ph.D.[2]
[1] Beckman Coulter Life Sciences, [2] Twist Bioscience

OVERVIEW

Targeted enrichment of next generation sequencing libraries allows for a cost-effective focus on regions of interest when compared to whole genome sequencing. Hybridization-capture based enrichment is one of the most utilized technologies for targeted enrichment. While hybridization-capture based enrichment can provide a high degree of uniformity and variant detection, it typically involves complex, labor-intensive workflows that can take several days to complete. In this poster, we describe the automation of Twist Bioscience's highly modular library preparation and hybridization-capture targeted enrichment chemistries with the use of the Twist Human Core Exome panel on the Beckman Coulter Biomek i7 Hybrid liquid handling platform. The method is capable of preparing up to 96 high quality NGS libraries from human genomic DNA using either enzymatic or mechanical fragmentation workflows and either Twist's Combinatorial Dual Indices or Universal Adapter System. Libraries can be enriched as either a single plex or pooled together up to an 8-plex using either Twist's standard or fast hybridization targeted enrichment protocols, resulting in enriched libraries that are ready to be sequenced on Illumina sequencing platforms. All major processes are performed on the system, allowing for maximum user walk away time and a workflow that can be performed in as little as a single day.

MATERIALS AND METHODS

The Biomek i7 Hybrid liquid handling system 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 HD752) and Twist Library Prep EF Kit with the Twist Universal Adapter System (Twist Bioscience Cat # 101059, 101308). 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 (Twist Cat # 102026, 100578) and dried down-off deck using a Vacufuge™ (Eppendorf™) set to room temperature. Dried down pools were hybridized for 2 hours and subsequent enrichment was performed using the Twist Fast Hybridization and Wash Kit (Twist Cat # 101174). Additionally, manually prepared libraries from Twist were enriched using the Twist Standard Hybridization and Wash Kit (Twist Cat # 101025) 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. Libraries were sequenced on an Illumina Next Seq 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).

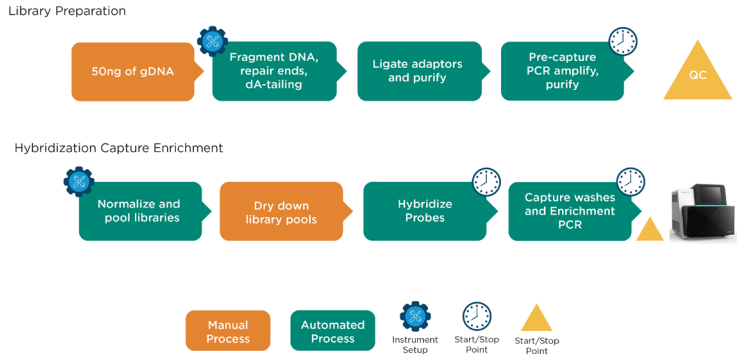


Figure 1. Example workflow of the automated Twist library preparation and enrichment on the Biomek i7 Hybrid liquid handling platform with user touch points and required manual user intervention steps highlighted.

RESULTS

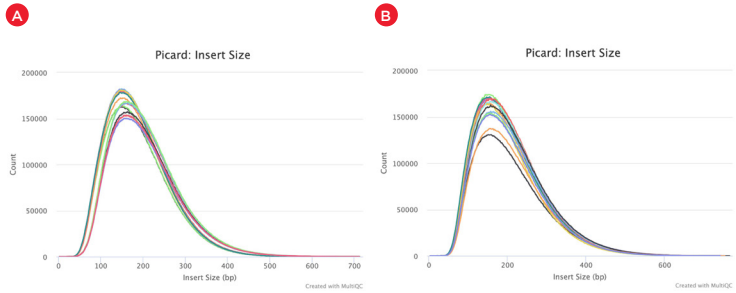


Figure 2. Twist Library Prep and Enrichment chemistries produce optimized, highly uniform multiplexed library pools on the Biomek i7. Traces display insert size along the x-axis vs. insert size count along the y-axis. Target mean insert size for sequencing was 180 bp. **(A)** Insert size sequencing data distribution for 16 libraries generated from the Twist Target Enrichment Protocol. Average insert size was 183 bp. **(B)** Insert size sequencing data distribution for 16 libraries generated from the Twist Fast Hybridization Target Enrichment Protocol. Average insert size was 188 bp.

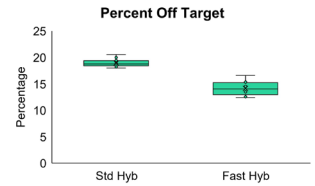


Figure 3. Low percentage of off targets observed in libraries enriched with Standard and Fast Hybridization chemistries on the Biomek i7. Per Twist specifications, off target should be below 25%. Mean percent off target is 18.9% for libraries enriched with the Standard Hybridization and Wash kit and 14.1% for libraries enriched with the Fast Hybridization and Wash kit.

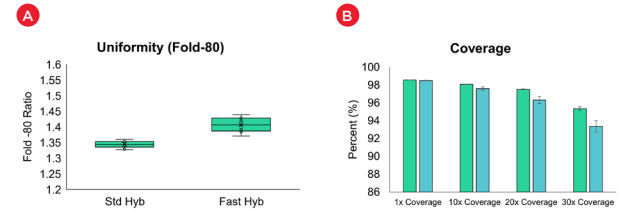
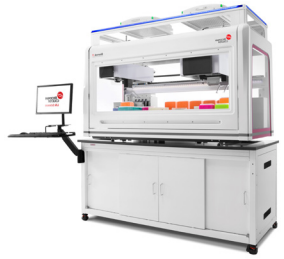


Figure 4. Automated Twist Enrichment produces highly uniform libraries and a high degree of sequencing depth. (A) Highly uniform libraries were prepared from both the Twist Standard and Fast Hybridization and Wash kits with a Fold-80 of 1.34 and 1.41, respectively. Expected Fold-80 ratios should be below 1.5. **(B)** Over 90% of the targeted regions were covered to a depth of at least 30X for both sets of samples enriched with the Twist Standard and Fast Hybridization and Wash kits.

CONCLUSIONS



In this study, we have shown consistent insert sizes (Figure 2), low percent off-target reads (Figure 3), a high degree of sequencing uniformity (Figure 4A) and high coverage (Figure 4B) for both Twist Fast and Standard Hybridization and Wash kits.

In conclusion, we have demonstrated a complete library preparation and enrichment workflow using Twist's NGS chemistries on the Biomek i7 Hybrid Liquid Handling 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.

For Twist NGS protocols used in this work, see **Twist's Technical Resources section** at **twistbioscience.com**.