Automated QIAseq® Targeted DNA Panel on Biomek i7 Hybrid Automated Workstation

Introduction

The QIAseq® Targeted DNA Panels enable Sample to Insight®, targeted next-generation sequencing (NGS) of DNA. Target enrichment technology enhances DNA NGS by enabling users to sequence specific regions of interest (ROI) – instead of the entire genome – which effectively increases sequencing depth and sample throughput while minimizing cost. Many commercially available target enrichment, library preparation, and sequencing methods use DNA polymerase and amplification processes that introduce substantial bias and artifacts. The QIAseq® Targeted DNA Panels overcome these biases/artifacts by utilizing a highly optimized reaction chemistry whereby unique molecular indices (UMIs) are integrated into a single gene-specific or ROI-specific, primer-based targeted enrichment process.

This highly optimized solution facilitates ultrasensitive variant detection from cells, tissue, and biofluids. The required amount of template for a single QIAseq® targeted sequencing reaction ranges from 10 to 80 ng for fresh DNA or 100 to 250 ng for formalin-fixed paraffin-embedded (FFPE) DNA.

This automated method on the Biomek i7 Hybrid workstation provides:

- Reduced hands-on time and pipetting errors
- Reduced chance of cross contamination
- Knowledgeable support from QIAGEN and Beckman Coulter Life Sciences

Figure 1. Biomek i7 Hybrid Automated Workstation.

Automated method

The automated QIAseq® Targeted DNA Panel method is constructed in a modular fashion and can be run start to finish with full walk-away capability. PCR reactions can be optimized and performed on-deck with an integrated thermocycler or with an off-deck thermocycler. The QIAseq® Targeted DNA Panel for the Biomek i7 automated method represents a complete implementation of all options allowed by the manual protocol including:

- Ability to process any number of samples from 1 to 96
- Flexibility with sample volumes or type, including either fresh DNA (10-80 ng standard genomic DNA or cfDNA), or FFPE DNA (100-250 ng)
- Variable sample input volume from 1 to 15 µL

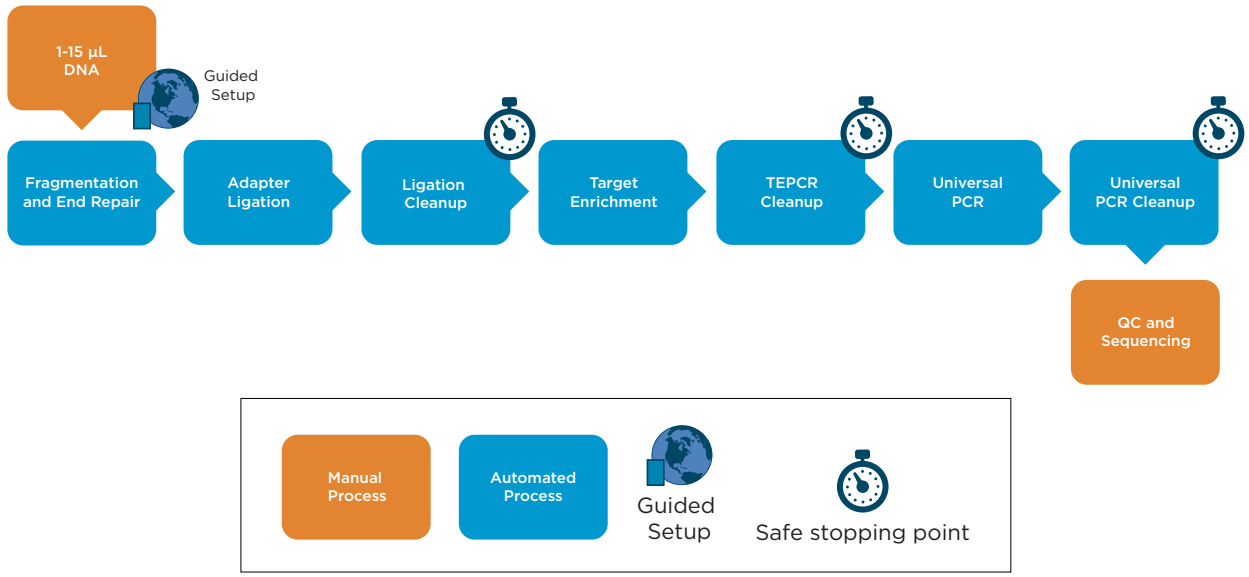

Figure 2. QIAseq® Targeted DNA Panel Workflow Diagram.

Automation provides increased efficiency and reduction in human errors, with minimal hands-on time. Users can start with DNA and end with sequence-ready libraries in just over 9 hours.

Section #	Automated Section Name	Time Duration
1	Fragmentation, Ligation and Cleanup	3 hours, 30 minutes
2	Target Enrichment and Cleanup	3 hours, 10 minutes
3	Universal PCR and Cleanup	2 hours, 30 minutes
	Total Time (with on-deck ATC)	**9 hours, 10 minutes**

Table 1. Estimated run time for automating QIAseq® Targeted DNA Panel for the Biomek i7 automated workstation.
*Total time estimates do not include reagent thawing or preparation. All thermocycler steps were performed on-deck.

Experimental Design

To demonstrate capabilities, a 24-sample plate of 10 ng of input DNA (Seraseq® Myeloid Mutation DNA Mix, 0710-0408) was compiled. The reagents for the automated method included the QIAseq® Targeted DNA Panel (333505) and the Human Myeloid Neoplasms Panel (DHS-0003Z). The quality of the libraries was assessed using Agilent TapeStation (High Sensitivity D1000 tape). The 24 libraries prepared with the QIAseq® Targeted DNA Panel Biomek i7 Hybrid automated method showed the library peak around 400 bps and no cross contamination in negative controls (NTC) **(Figure 3)**. Qubit™ analysis (1X hsDs DNA) was performed for quantification. Four libraries were randomly selected for sequencing. The libraries were diluted to 4 nM concentration, denatured, and loaded at 10 pM on the MiSeq® (Illumina©) using the MiSeq® Reagent Kit (v2 300-cycle). All sequencing analysis was performed using the QIAGEN GeneGlobe® Design and Analysis Hub.

Results

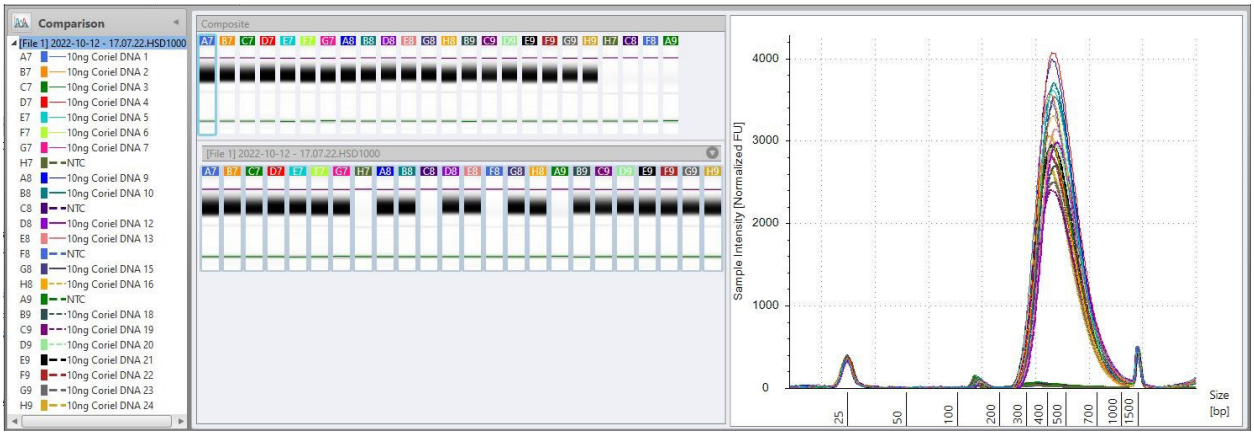

Figure 3. Library yield analyzed on TapeStation 2200 with High Sensitivity D1000 tape. Traces show overlay with negative controls (NTC).

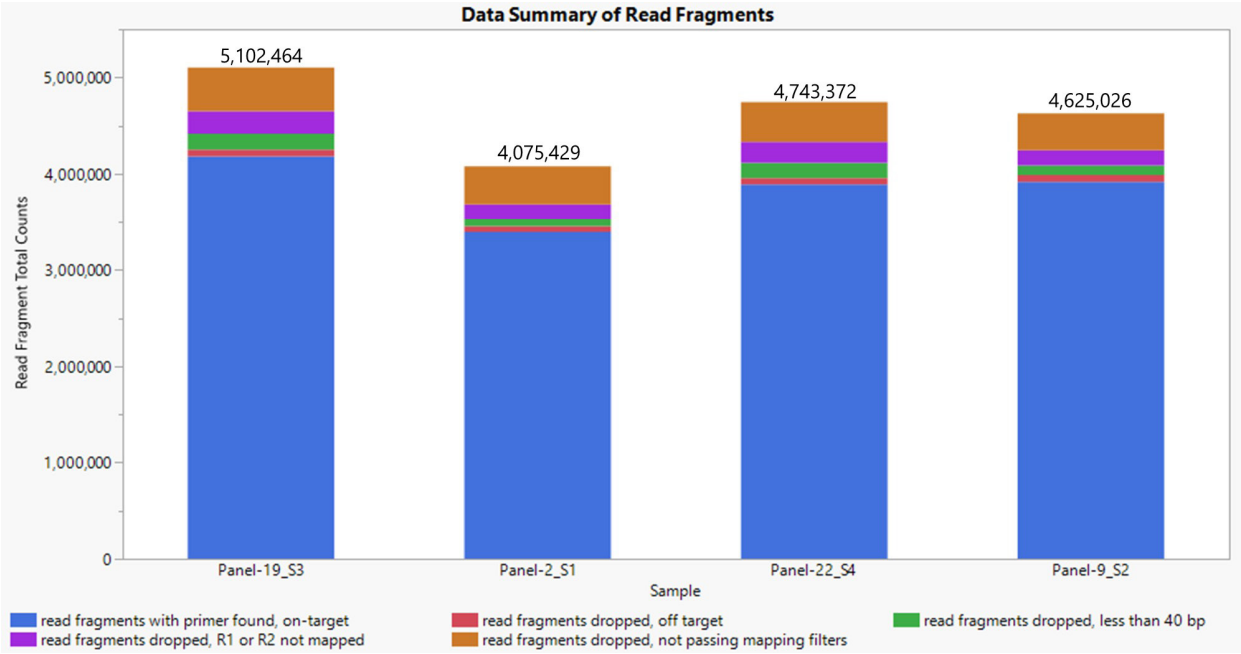

Figure 4. Alignment statistics for each of the QIAseq® Targeted DNA Panel libraries sequenced. The total number of reads is displayed above the stacked bar for each sample. This graph shows the classification breakdown for all fragment reads. The number of read fragments aligned to the targeted sequence contributed to over 98% of the total read fragment count.

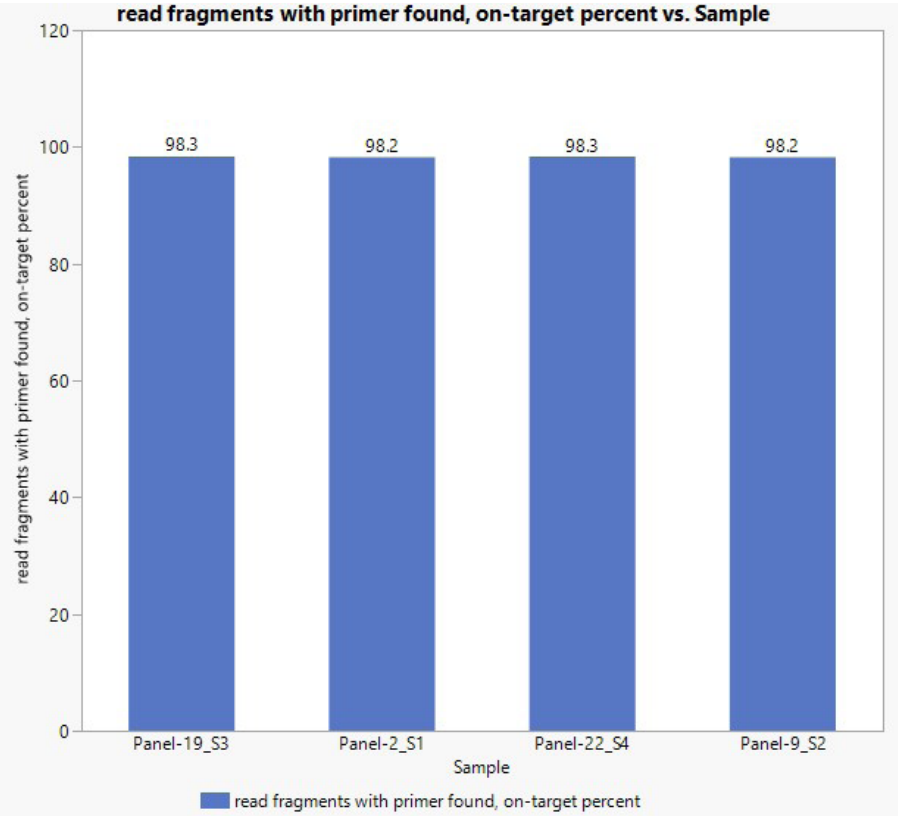

Figure 5. Bar graph displaying the % fragment reads aligned to the target sequence. Read Fragments for the libraries generated with the QIAseq® Targeted DNA Panel Automated Method for the Biomek i7 Hybrid Automated Workstation aligned to the target sequence with a success rate of over 98%.

Summary

We have demonstrated that the automated workflow for the QIAseq® Targeted DNA Panel from fragmentation to final library amplification cleanup can be done in 9.5 hours for 24 samples on the Biomek i7 Dual Hybrid Workstation. The workflow provides the ability for variable input amounts, sample types, mass, and volumes. It also provides on-deck or off-deck thermocycler options, as well as customer index plate and automatic or customer target enrichment PCR product split transfer options. This automated method provides the flexibility and completely walk-away solution for QIAseq® Targeted DNA Panel Library Preparation with high quality of libraries for downstream sequencing analysis with no cross contamination in the plate.

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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