



## Automated QIAseq® Targeted DNA Pro Panels on the Biomek i7 Hybrid Liquid Handler

### Introduction

The QIAseq Targeted DNA Pro Panels enable streamlined 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. The QIAseq Targeted DNA Pro Panels overcome biases/artifacts by utilizing a highly optimized reaction chemistry by incorporating unique molecular indices (UMIs) into a single gene or ROI-specific, primer-based targeted enrichment process. By replacing the bead cleanups with enzymatic cleanups after the ligation and target enrichment steps, the QIAseq Targeted DNA Pro Panels enable a more efficient, quick, consistent, and automation-friendly workflow.

This highly optimized, automation-friendly solution facilitates ultrasensitive variant detection from cells, tissue, and biofluids within hours. The required amount of template for a single QIAseq Targeted DNA Pro library prep reaction ranges from 10 to 80 ng of fresh DNA, or 100 to 250 ng of formalin-fixed paraffin-embedded (FFPE) DNA. The QIAseq Targeted DNA Pro Panels incorporate a seamless FFPE DNA repair step before library construction, combined with QIAseq target enrichment technology, result in great improvements on the recovery of FFPE DNA samples.

Compared to manual pipetting, when automated on Biomek i7Hybrid workstation, the Illumina-compatible QIAseq® Targeted DNA Pro Panels provide:

- Reduced hands-on time and increased throughput
- Reduction in pipetting errors
- Standardized workflow for improved results
- Knowledgeable support from QIAGEN and Beckman Coulter Life Sciences

## Spotlight

The Biomek i7 Hybrid automated workstation delivers reliability and efficiency to increase user confidence and walk-away time compared to manual operation.

The features of the Biomek i7 Liquid Handler include:

- 300  $\mu$ L or 1200  $\mu$ L Multichannel head with 1-300  $\mu$ L and 1-1200  $\mu$ L pipetting capability
- Span-8 pod with disposable tips
- Enhanced Selective Tip pipetting to transfer custom arrays of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with 45 positions
- Shaking and temperature-controlled positions for sample processing (not included with the system)
- Spacious open platform design to integrate on-deck and off-deck elements (e.g. thermocyclers)



**Figure 1.** Biomek i7 Hybrid Liquid Handler.

## Automated method

The QIAseq® Targeted DNA Pro automated method is constructed in a modular fashion and can be run start to finish with full walk-away capability. Alternatively, the automated method can be processed as 3 different method sections, providing users flexibility in scheduling their workflow. Each method section ends with a QIAGEN supported safe stop point. The user can start with DNA and end with sequencing-ready libraries in under 7 hours.

Section #	Section Description	Time Duration
1	Fragmentation	50 minutes
	Ligation	45 minutes
	Ligation Cleanup	40 minutes
	Target Enrichment	1 hour, 5 minutes
<b>Total Time for Section 1</b>		<b>3 hours, 20 minutes</b>
2	Target Enrichment Cleanup	45 minutes
	Universal PCR	1 hour, 10 minutes
<b>Total Time for Section 2</b>		<b>1 hour, 55 minutes</b>
3	Universal PCR Cleanup	50 minutes
<b>Total Time for Section 3</b>		<b>50 minutes</b>
<b>Total Time (with on-deck ATC)</b>		<b>6 hours, 5 minutes</b>

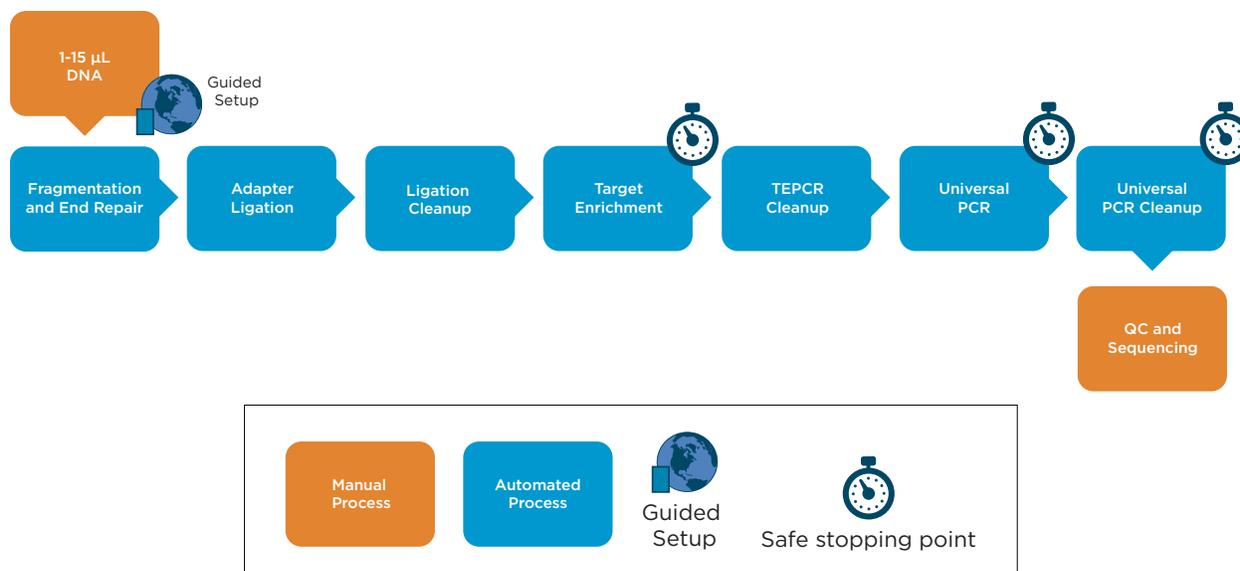
**Table 1.** Estimated runtime for QIAseq® Targeted DNA Pro automated method for the Biomek i7 Hybrid Automated Workstation.

\*Total time estimates do not include reagent thawing or preparation.

\*\*Time estimates were calculated on a 24-sample automated run.

The features of the QIAseq® Targeted DNA Pro for Biomek i7 automated method include:

- Ability to process any number of samples (1-96)
- User-defined starting material and concentration of either:
  - Standard genomic DNA (10-80 ng)
  - cfDNA (10-80 ng)
  - or FFPE DNA (100-250 ng)
- Support for either on-deck or off-deck thermocycler
- Variable sample input volume (1-9 µL) or panel count (<12,000 or ≥12,000)
- Support for original index plate or custom index input
- Automatic or custom target enrichment PCR (TEPCR) product transfer
- Optimization of PCR cycles for TEPCR (24-36 cycles)
- Variable ethanol drying time (1-20 minutes)

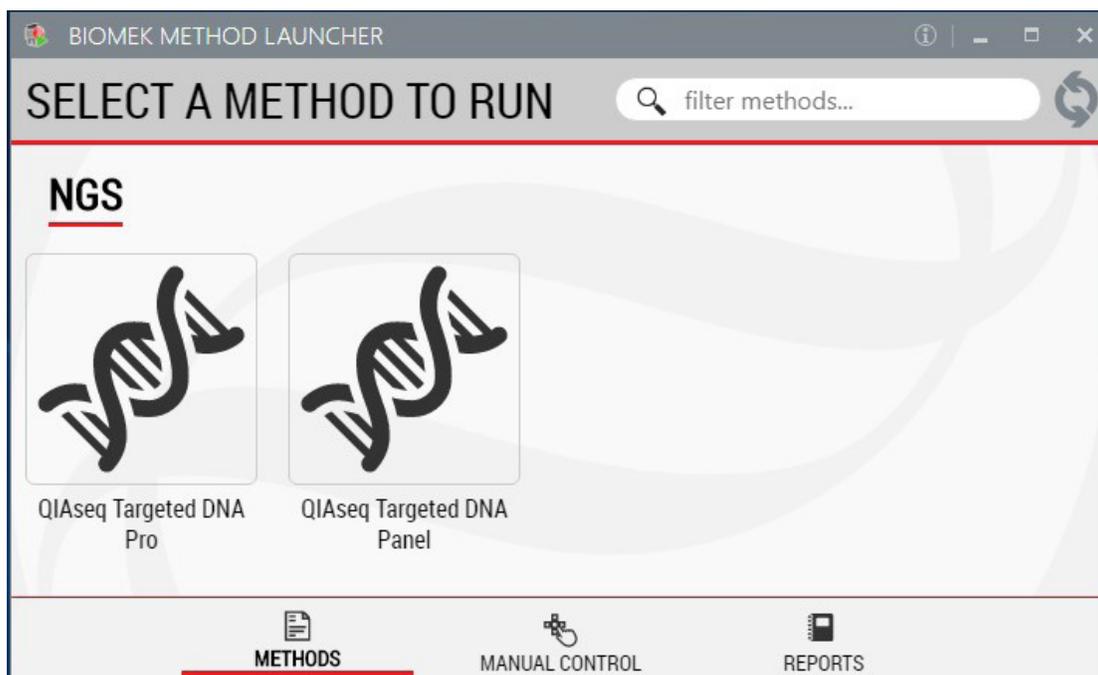


**Figure 2.** QIAseq® Targeted DNA Pro Workflow Diagram.

The software provides several user-friendly features, such as:

1. Biomek Method Launcher (BML):

BML is a secure interface for method implementation that prevents compromising method integrity. It allows users to remotely monitor the progress of the run. It also features DeckOptix Final Check software to reduce deck setup errors, and thus prevent failed experiments due to missing or misplaced labware and incorrect tip or plate type. The manual control options provide the opportunity to interact with the instrument, if needed.



**Figure 3.** Biomek Method Launcher interface.

## 2. Method Option Selector (MOS)

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

### QIAGEN QIAseq<sup>®</sup> Targeted DNA Pro

Optimized for Biomek i7 Dual Hybrid Automated by Beckman Coulter

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#### Method Parameters

Samples Type:  ▾  
Number of Samples:  (1-96)  
Use On-Deck Thermocycler?  ▾

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#### Method Options

**Fragmentation, Ligation and Target Enrichment (Safe Stop)**  
Sample input volume (μL):  (1-9)  
Number of primers per tube in the panel:  ▾

**TEPCR Cleanup and Universal PCR (Safe Stop)**  
Manually aliquot 20μL of TEPCR reaction off-deck?  ▾  
Manually aliquot indices to a new plate off-deck?  ▾  
Index plate starting position:  ▾  
Number of universal PCR cycles:  ▾

**Universal PCR Cleanup (Safe Stop)**  
Ethanol drying time (min):  (1-20)



**Figure 4.** Method Option Selector (MOS) enables users to select sample type, sample number, on- or off-deck thermocycler location, and workflow options processing.

### 3. Guided Labware Setup (GLS)

GLS provides the user with a graphical setup, reagent volume calculations, and step-by-step instructions to prepare master mixes. The specific setup of the deck changes to accommodate the specific method parameters chosen in the MOS.

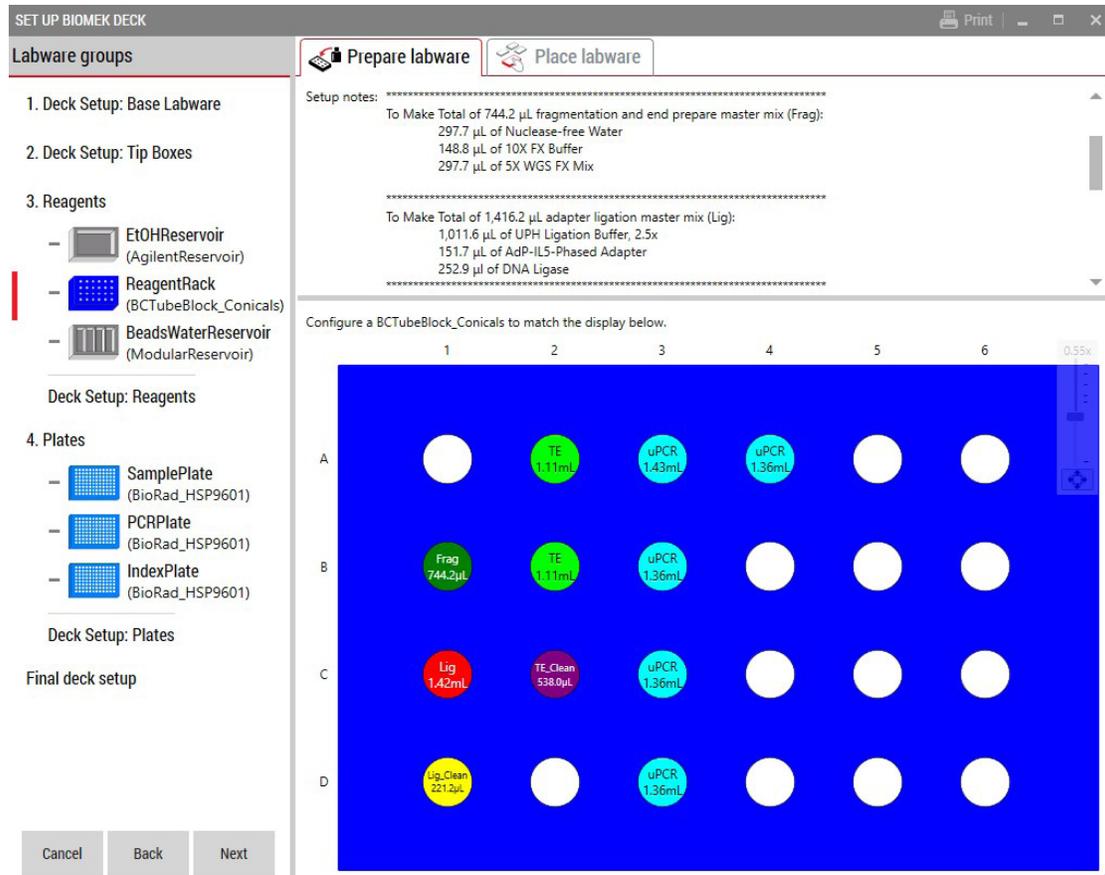
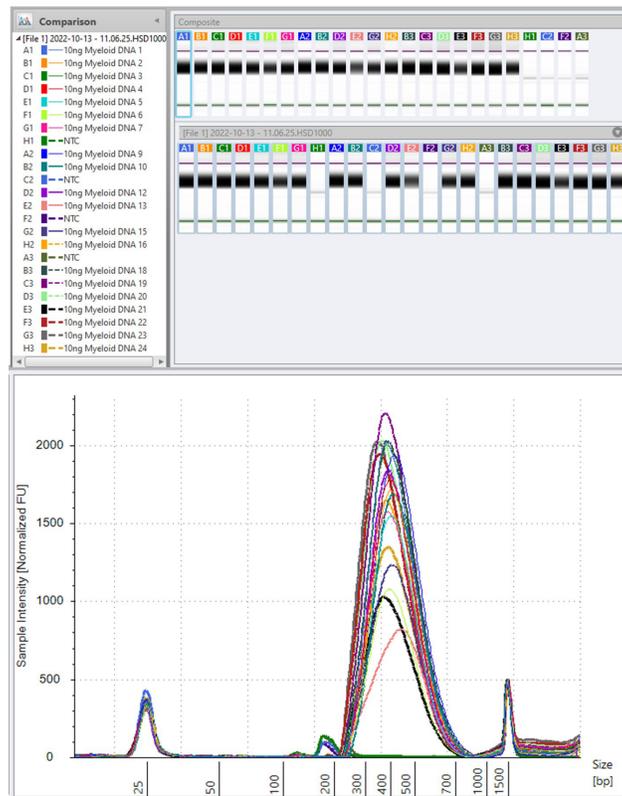


Figure 5. Guided Labware Setup displays reagent volumes and guides the user for correct deck setup.

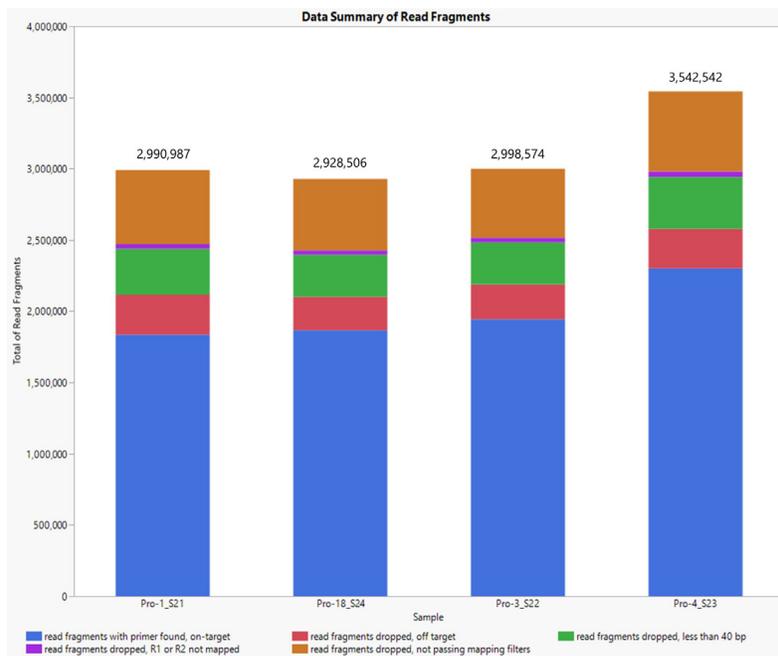
## Experimental Design

To demonstrate capabilities, a 24-well plate consisting of 20 replicates of 10 ng of DNA (Seraseq<sup>®</sup> Myeloid Mutation DNA Mix, 0710-0408) and 4 non-template controls (NTC) was utilized for library construction. The reagents for the automated chemistry run included the QIAseq<sup>®</sup> Targeted DNA Pro kit (333655), QIAseq<sup>®</sup> Targeted DNA Pro UDI Set A (333455), and the Myeloid Neoplasms Focus Panel (PHS-103Z-96). The quality of the libraries was assessed using with Agilent TapeStation chemistry (High Sensitivity D1000 tape) and samples were quantified with 1X hsDs DNA Qubit<sup>™</sup>. A random selection of four libraries were diluted down to 4 nM concentration, denatured and loaded at 10 pM on the MiSeq<sup>®</sup> (Illumina<sup>®</sup>) and sequenced using the MiSeq<sup>®</sup> Reagent Kit (v2 300-cycle). All sequencing analysis was performed using the QIAGEN GeneGlobe<sup>®</sup> Design and Analysis Hub.

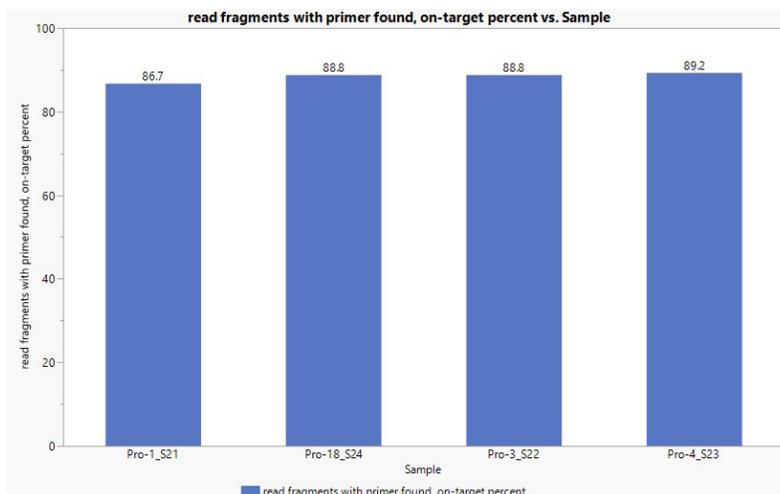
## Results



**Figure 6.** Library yield analyzed on TapeStation 4200 with High Sensitivity D1000 screening tape. Traces are overlaid with non-template controls (NTC).



**Figure 7.** Alignment statistics for each of the QIAseq® Targeted DNA Pro 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 total number of read fragments exceeded 2.9M and the percentage aligned to the targeted sequence attributed to over 86% of the total read fragment count.



**Figure 8.** Bar graph displaying the % fragment reads aligned to the target sequence. Read fragments for the libraries generated with the QIAseq® Targeted DNA Pro Automated Method for the Biomek i7 Hybrid Automated Workstation aligned to the target sequence with a success rate of over 86%.

## Summary

We have demonstrated that the automated workflow for the QIAseq® Targeted DNA Pro Panels from fragmentation to final library can be done in under 7 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 Pro Library Preparation with high quality of libraries for downstream sequencing analysis.

The QIAseq® Targeted DNA Pro Library Prep kit is for Research Use Only. The QIAseq® Targeted DNA Pro Library Prep kit is 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 intended or validated for use in the diagnosis of disease or other conditions.

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