Automating QIAseq® DIRECT enhanced SARS-CoV-2 for Biomek i7 Hybrid Automated Workstation

Introduction

The QIAseq® DIRECT enhanced SARS-CoV-2 Kit is specially designed to aid in variant detection and estimation of epidemiological prevalence of the SARS-CoV-2 virus, which is the causative agent of coronavirus disease 2019 (COVID-19). The size of the entire SARS-CoV-2 virus genome is under 30 kb and can be mixed with host RNA when isolating from a human sample, making it challenging to reconstruct the whole genome of the virus. While next-generation sequencing (NGS) has become a vital tool, streamlined library preparation solutions remain elusive for SARS-CoV-2 assessment. The QIAseq® DIRECT enhanced SARS-CoV-2 Kit represents a rapid library prep, enabling high-throughput, whole genome enrichment of SARS-CoV-2 for mutation surveillance by NGS.

The QIAseq® DIRECT enhanced SARS-CoV-2 Biomek i7 Hybrid automated method begins with 5µL of viral RNA and can prepare up to 96 sequence-ready libraries with an estimated completion time of 7 hours and 35 minutes. This automated method can also split into 1-3 days in accordance with approved safe stop points. In this application note, we will demonstrate the performance of the QIAseq® DIRECT enhanced SARS-CoV-2 on the Biomek i7 Hybrid Automated Workstation System.

The QIAseq® DIRECT enhanced SARS-CoV-2 Biomek i7 Hybrid automated method provides:

- Reduced hands-on time and pipetting errors
- Quick installation with ready-to-implement method
- Knowledgeable support from QIAGEN and Beckman Coulter Life Sciences

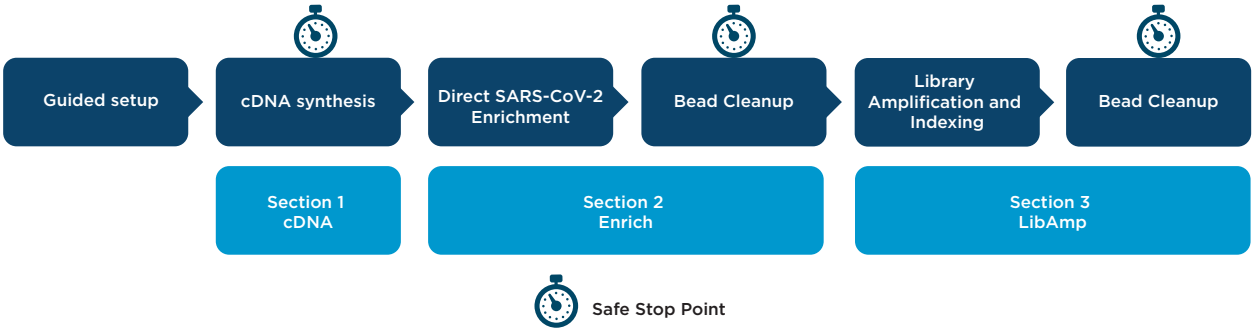

Figure 1. QIAseq® DIRECT enhanced SARS-CoV-2 automated workflow for the Biomek i7 Hybrid Automated Workstation System.

Spotlight

The Biomek i7 Hybrid Automated Workstation System features dual pipetting heads and flexible configurations to increase customer confidence and walk-away time. The workstation features include:

- Dual pipetting head with 1-1000µL pipetting capability
- Span-8 pod with disposable tips
- Independent 360˚ rotating grippers with offset fingers
- High-capacity deck with up to 45 positions
- Shaking and heating/cooling for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermocycler)

Figure 2. Biomek i7 Hybrid Automated Workstation.

Automated Method

Automation provides increased efficiency and reduction in human errors, with minimal hands-on time **(Table 1)**.

Sample Number	48	96
Instrument Setup Time	15 minutes	20 minutes
Method Run Time; Section 1	1 hour, 30 minutes	1 hour, 30 minutes
Method Run Time; Section 2	3 hours	3 hours, 30 minutes
Method Run Time; Section 3	2 hours	2 hours, 15 minutes
Total Time (with on-deck ATC)	6 hours, 45 minutes	7 hours, 35 minutes

*Total time estimates do not include reagent thawing and preparation.

Table 1. Estimated run time for automating QIAseq® DIRECT enhanced SARS-CoV-2 on the Biomek i7 Hybrid Automated Workstation.

The method can be run using the Method Option Selector **(Figure 3)**, Guided Labware Setup to aid with deck setup and reagent calculations, and DeckOptix Final Check software to minimize costly setup errors. Automated methods provide flexibility to users in scheduling their workflow and allowing method customizations for sample processing and throughput.

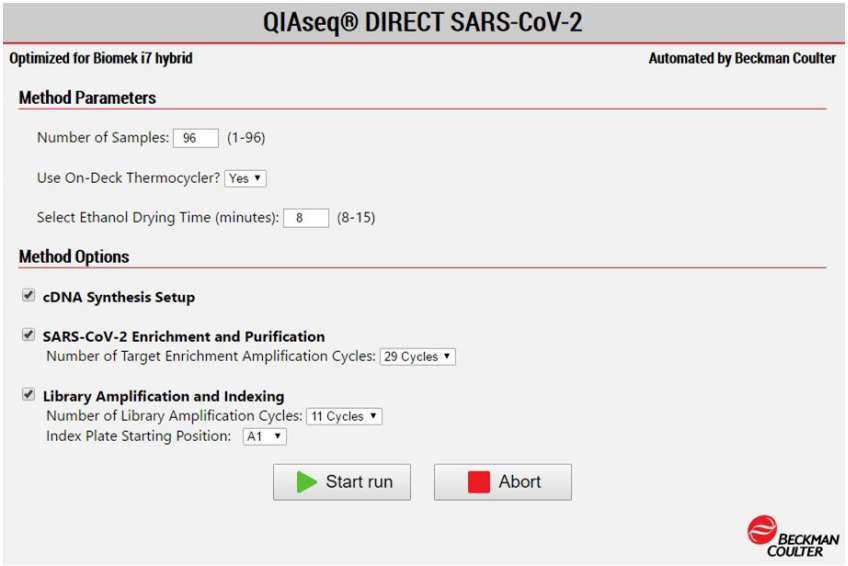

Figure 3. QIAseq® DIRECT enhanced SARS-CoV-2 automated Method Option Selector (MOS) enables users to select sample number, On- or Off-Deck Thermocycler, Ethanol drying time, and method options for index locations and amplification cycles.

Experimental design

To demonstrate method capabilities for a 96-sample run QIAseq® DIRECT enhanced SARS-CoV-2 libraries were prepared on a Biomek i7 Hybrid automated workstation. We selected 5 different extractions from nasopharyngeal swabs positive for the presence of the SARS-CoV-2 virus and placed them in replicates of 16 across a 96-well plate. A total of 8 replicates of a SARS-CoV-2 positive control (ATCC, #VR-3347D) and 8 replicates of a Negative Template Controls (NTCs) were added to make a complete sample set of 96 depicted in Figure 4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	Sample1	Sample2	Sample3	Sample4	Sample5	NTC	Sample1	Sample2	Sample3	Sample4	Sample5
B	Pos Ctrl	Sample1	Sample2	Sample3	Sample4	Sample5	Pos Ctrl	Sample1	Sample2	Sample3	Sample4	Sample5
C	Sample1	Sample2	NTC	Sample3	Sample4	Sample5	Sample1	Sample2	NTC	Sample3	Sample4	Sample5
D	Sample1	Sample2	Pos Ctrl	Sample3	Sample4	Sample5	Sample1	Sample2	Pos Ctrl	Sample3	Sample4	Sample5
E	Sample1	Sample2	Sample3	Pos Ctrl	Sample4	Sample5	Sample1	Sample2	Sample3	Pos Ctrl	Sample4	Sample5
F	Sample1	Sample2	Sample3	NTC	Sample4	Sample5	Sample1	Sample2	Sample3	NTC	Sample4	Sample5
G	Sample1	Sample2	Sample3	Sample4	Sample5	Pos Ctrl	Sample1	Sample2	Sample3	Sample4	Sample5	Pos Ctrl
H	Sample1	Sample2	Sample3	Sample4	Sample5	NTC	Sample1	Sample2	Sample3	Sample4	Sample5	NTC

Figure 4. Schematic representation of the sample plate setup.

Final libraries analyzed with High Sensitivity DNA ScreenTape (Agilent, #5067-5584 and 5067-5585). The median insert size for the SARS-CoV-2 positive control was 407 ± 18 bps and 403 ± 38 bps for the nasopharyngeal samples **(Figure 5)**. All NTCs resulted in no yield, while library concentrations from the SARS-CoV-2 positive control averaged 3.9ng/µL for all replicates (4.3ng/µL for sequencing selections), and 0.97 ng/µL for all nasopharyngeal samples (1.6ng/µL for sequencing selections), **(Figure 6)**. A selection of 10 libraries (2 Positive Controls, and 1-2 libraries from each nasopharyngeal sample) were normalized and pooled. Data analysis was conducted using QIAGEN's SARS-CoV-2 CLC genomics workbench plugin.

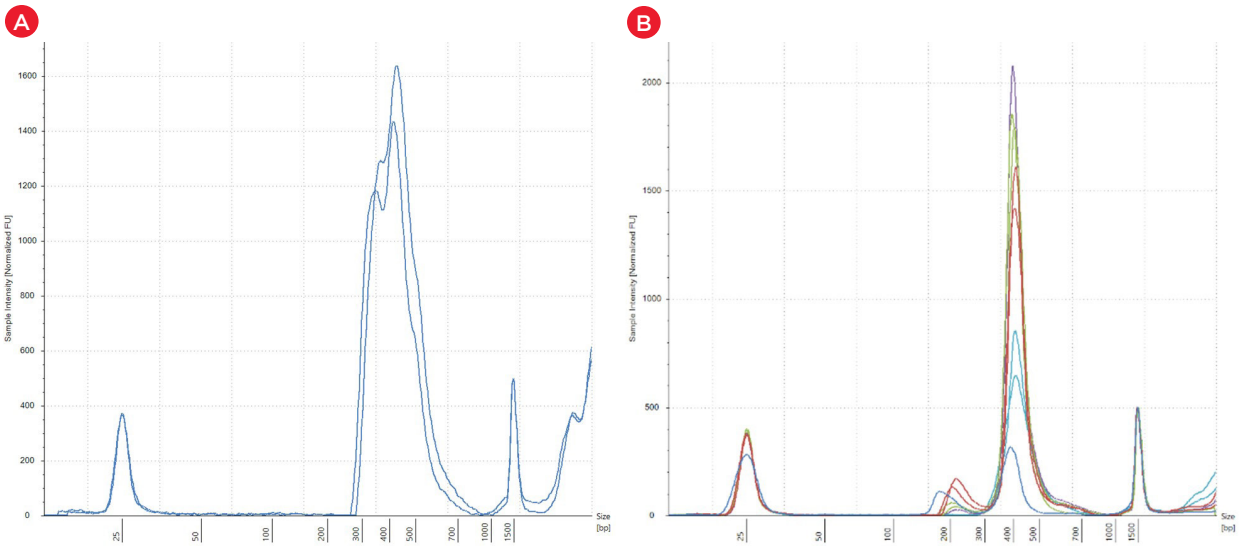

Figure 5. TapeStation High Sensitivity electropherogram data for Biomek i7 Hybrid automated method for QIAseq® DIRECT enhanced SARS-CoV-2. The electropherogram compilation of libraries selected for sequencing from SARS-CoV-2 positive controls **(Figure 5.a)** and nasopharyngeal samples positive for SARS-CoV-2 **(Figure 5.b)**.

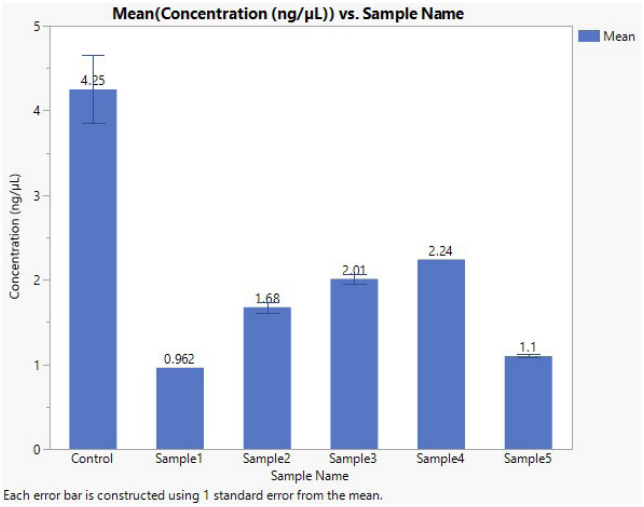

Figure 6. Mean concentration (ng/µL) of QIAseq® DIRECT enhanced SARS-CoV-2 libraries by the sample name. Libraries were created using the Biomek i7 Hybrid automated method.

Results

The completed pool was sequenced on an Illumina MiSeq using v3 chemistry. The mean percentage of mapped reads for the SARS-CoV-2 positive control was 99.6% and 85.2% for the nasopharyngeal samples **(Figure 7)**. Analysis of the nasopharyngeal sequencing reads demonstrated even genomic distribution across the NC_045512 SARS-CoV-2 gene **(Figure 8)**.

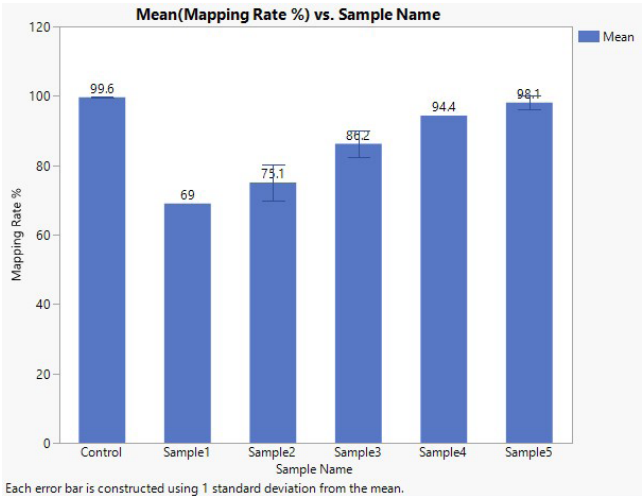

Figure 7. Mean mapping rate % by sample name of the Biomek i7 Hybrid automated method for the QIAseq® DIRECT enhanced SARS-CoV-2.

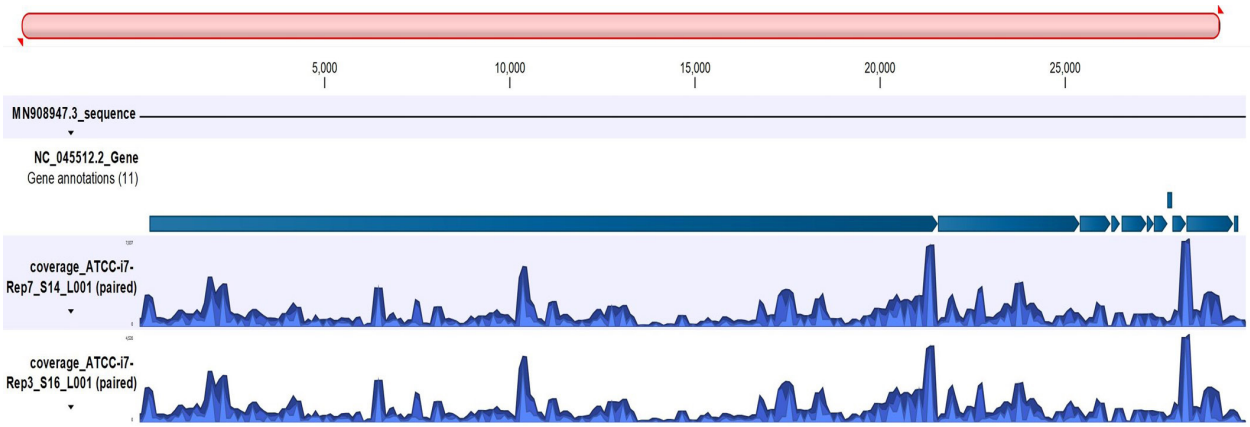

Figure 8. Visual representation of the SARS-CoV-2 genome showing high coverage across the whole genome. Data was generated from the Biomek i7 Hybrid automated method for QIAseq® DIRECT enhanced SARS-CoV-2 and analyzed using QIAGEN's CLC genomic workbench.

Summary

We've demonstrated that the automation of QIAseq® DIRECT enhanced SARS-CoV-2 on the Biomek i7 Hybrid Automated Workstation provides an efficient, flexible, and scalable solution for any size lab. The automation solution delivers libraries that yield quality results in downstream workflows and saves valuable time and resources.

References

QIAGEN. (2022). *DIRECT SARS-CoV-2.* Retrieved from **https://www.qiagen.com/us/Resources/ResourceDetail?id=51a9f15a-8a8c-45f9-882f-e30d8c52f6d9&lang=en/.**

Acknowledgments

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

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