Automating QIAseq® DIRECT enhanced SARS-CoV-2 Kit on Biomek i5 Span-8 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 automated method begins with 5µL of viral RNA and can prepare up to 48 sequence-ready libraries with an estimated completion time of 6 hours and 15 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 Kit on the Biomek i5 Span-8 Automated Workstation System.

The QIAseq® DIRECT enhanced SARS-CoV-2 Biomek i5 Span-8 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

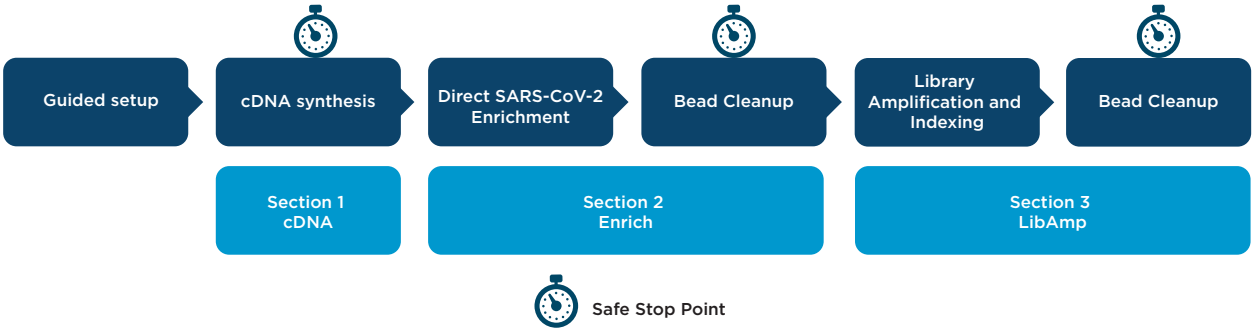

Figure 1. QIAseq® DIRECT enhanced SARS-CoV-2 automated workflow for the Biomek i5 Span-8 Automated Workstation System.

Spotlight

The Biomek i5 Span-8 Automated Workstation System features a Span-8 pipetting head and flexible configurations to increase walk-away time.

The workstation features include:

- Span-8 pod with disposable tips
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity with up to 25 positions
- Shaking and heating/cooling for controlling sample processing (not included with system)
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermocyclers

Figure 2. Biomek i5 Span-8 Automated Workstation.

Automated Method

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

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

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

Table 1. Estimated run time for automating QIAseq® DIRECT enhanced SARS-CoV-2 on the Biomek i5 Span-8 Automated Workstation.

The method can be run using Method Option Selector **(Figure 3)**, Guided Labware Setup to aid with deck setup and reagent calculations **(Figure 4)**, and DeckOptix Final Check software to minimize costly setup errors. Automated methods provide flexibility to users in scheduling their workflow and allowing method customizations sample processing and throughput.

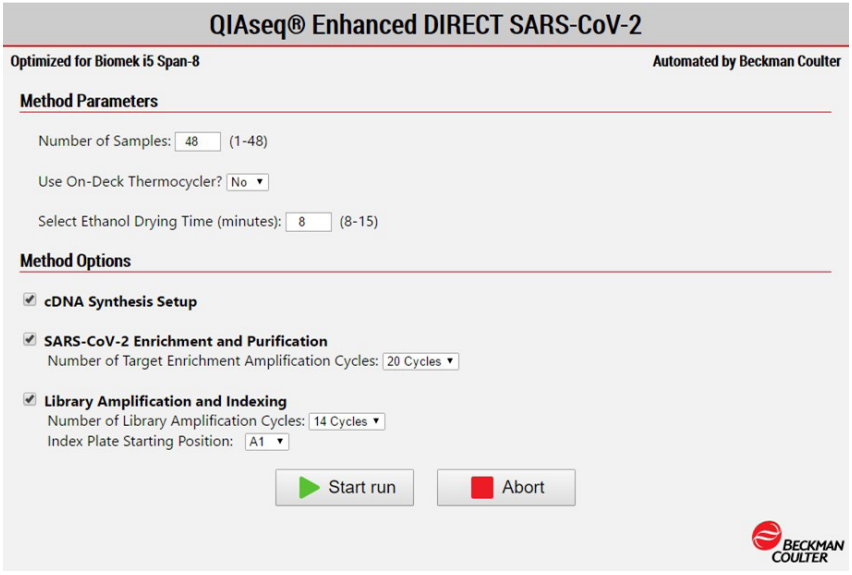

Figure 3. QIAseq® DIRECT enhanced SARS-CoV-2 automated Method Option Selector (MOS) enables users to select sample number, On- or Off-Deck Thermocycler, ethanol drying time, and method options for index locations and amplification cycles.

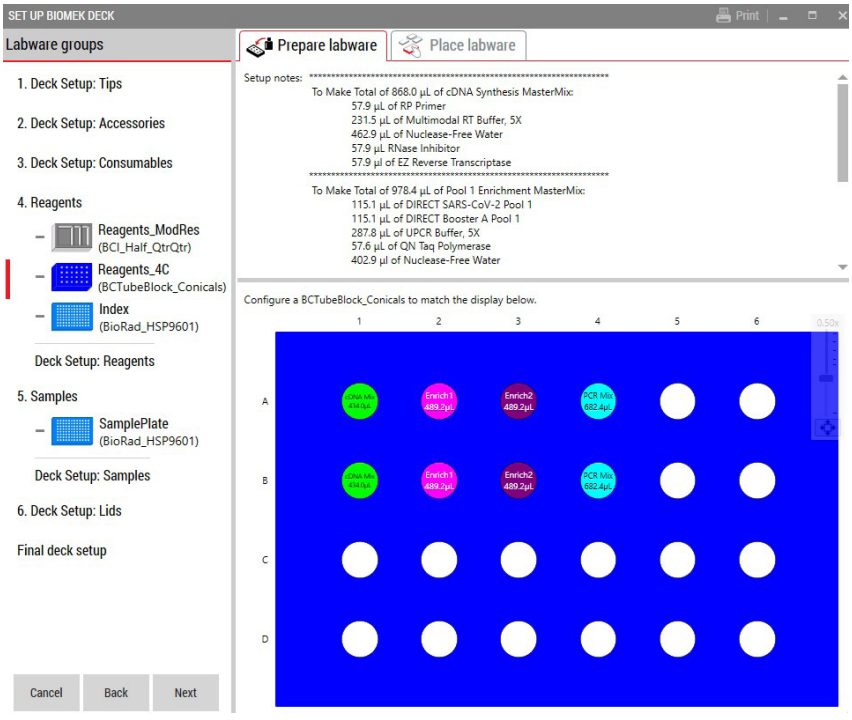

Figure 4. Guided Labware Setup (GLS) provides reagent volumes calculated by sample number, preparation notes, and pictorial guide for user deck setup.

Experimental Design

To demonstrate method capabilities for a 48-sample library preparation on a Biomek i5 Span-8 automated workstation, we selected 5 different extractions from nasopharyngeal swabs positive for the presence of the SARS-CoV-2 virus. The nasopharyngeal samples were placed in replicates of 8 across 48 wells of a 96-well plate. A total of 4 replicates of the SARS-CoV-2 positive control (ATCC, #VR-3347D) and 4 replicates of a Negative Template Controls (NTCs) were added to make a complete sample set of 48.

Final libraries analyzed with High Sensitivity DNA ScreenTape (Agilent, #5067-5584 and 5067-5585), **(Figure 5a and 5b)**. All NTCs resulted in no yield, while the mean library concentration from SARS-CoV-2 positive control was 14.4ng/µL and 21.5ng/µL from nasopharyngeal samples **(Figure 6)**. A selection of 10 libraries (2 Positive Controls, and 1-3 libraries from each nasopharyngeal sample) were normalized and pooled for sequencing. Data analysis was conducted using QIAGEN's SARS-CoV-2 CLC genomics workbench plugin.

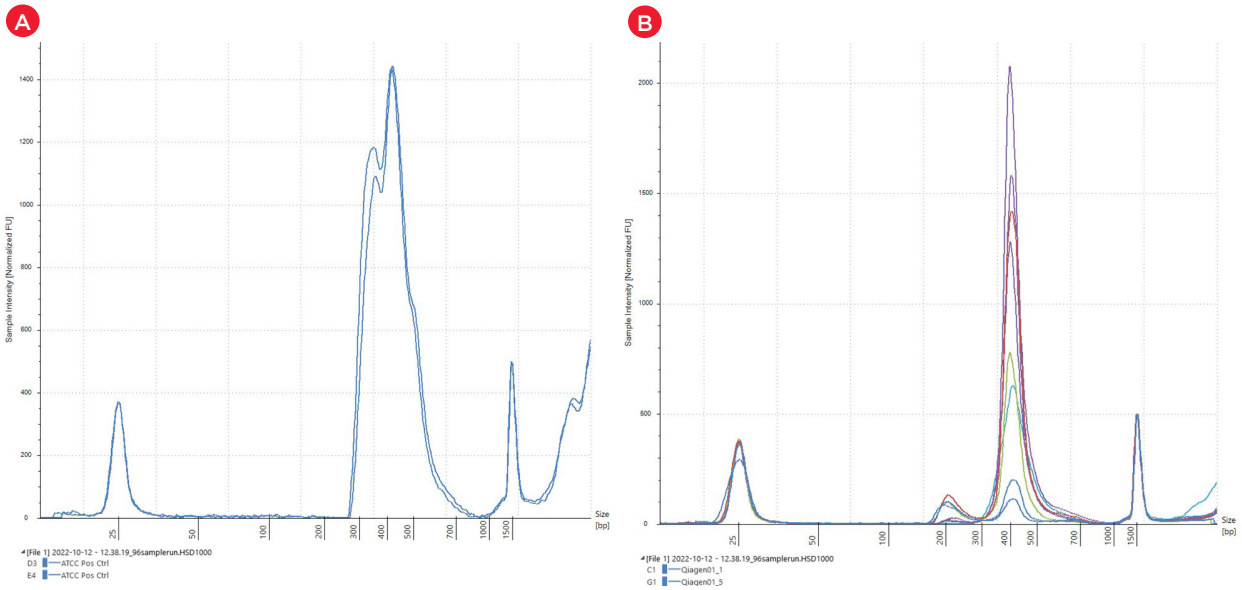

Figure 5. TapeStation High Sensitivity electropherogram data for automated Biomek i5 Span-8 method for QIAseq® DIRECT enhanced SARS-CoV-2. The electropherogram compilation of libraries selected for sequencing from SARS-CoV-2 positive controls **(Figure 5.a)** and nasopharyngeal samples positive for SARS-CoV-2 **(Figure 5.b)**.

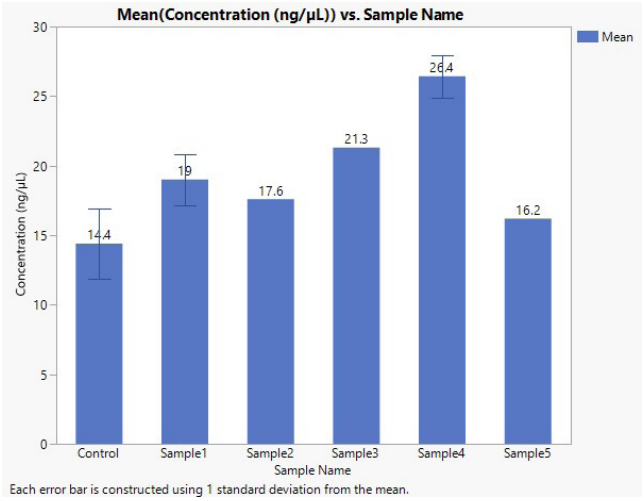

Figure 6. Mean concentration (ng/µL) by sample of libraries generated with the Biomek i5 Span-8 automated method for QIAseq® DIRECT enhanced SARS-CoV-2.

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 98.6% and 96.25% 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)**.

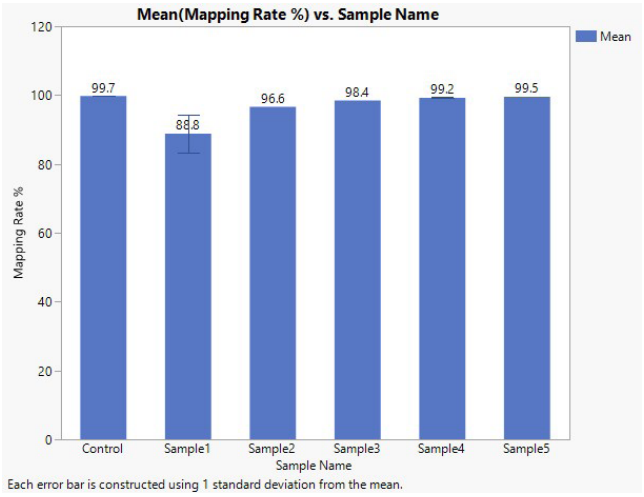

Figure 7. Mean mapping rate % by sample of libraries generated with the Biomek i5 Span-8 automated method for QIAseq® DIRECT enhanced SARS-CoV-2. The mean of the mapping rate percentages for the control libraries was 98.6% and the combined mean for all the nasopharyngeal samples was 96.25%.

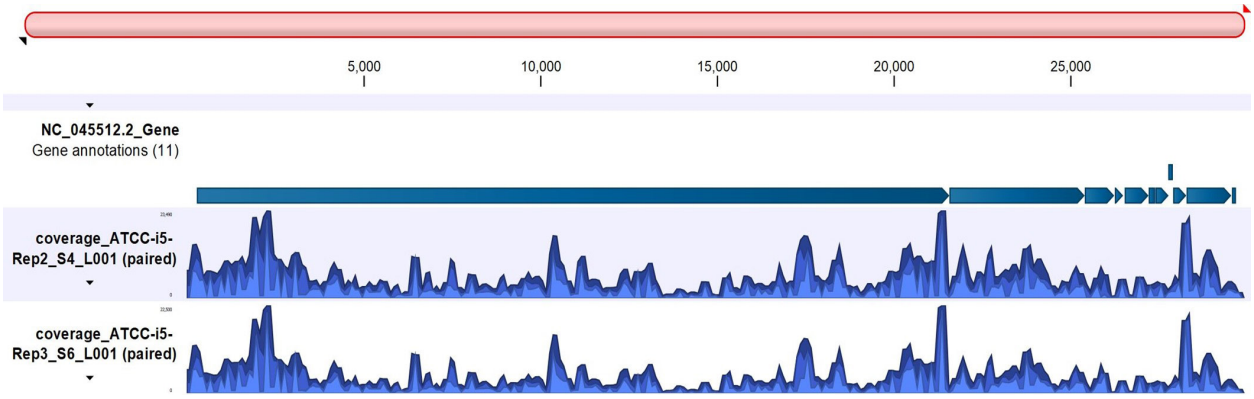

Figure 8. Visual representation of the SARS-CoV-2 genome demonstrating high coverage across the whole genome. Data was generated from the Biomek i5 Span-8 automated method for QIAseq® DIRECT enhanced SARS-CoV-2 and analyzed using QIAGEN's CLC genomic workbench.

Summary

We've demonstrated that the automation of QIAseq® DIRECT enhanced SARS-CoV-2 on the Biomek i5 Span-8 automated workstation provides an efficient, flexible, and scalable solution for labs of any size. 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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