BECKMAN COULTER
Life Sciences

Illumina RNA Prep, (L) Tagmentation with Enrichment on the Biomek i7 Hybrid

Introduction

The Illumina RNA Prep with Enrichment assay is a flexible target enrichment next generation sequencing (NGS) kit for use with targeted RNA sequencing and respiratory viral detection, including detection of the novel SARS-CoV-2 virus. The workflow allows for purified total RNA samples (10-100 ng input) or RNA from formalin-fixed paraffin embedded (FFPE) or degraded samples (20-100 ng input). RNA is reverse transcribed to complementary DNA (cDNA), which is then tagmented using Illumina's Enriched Bead-Linked Transposomes to create larger inserts and indexed using IDT for Illumina DNA/RNA Unique Dual (UD) indexes. Libraries can be processed through enrichment by pooling samples down to a 3-plex or as a single-plexed library. The targeted enrichment portion of the assay uses a single 2-hour hybridization to allow for rapid capture and enrichment of the library pools. The entire manual workflow from cDNA synthesis and library preparation to targeted enrichment can be performed in a single day.

In this application note, we describe and demonstrate the automated performance of the Illumina RNA Prep, (L) Tagmentation with Enrichment using the Illumina Respiratory Virus Oligos Panel v2 on the Biomek i7 Hybrid Genomics Workstation. The automated method can support cDNA synthesis and library construction between 1 to 96 samples and targeted enrichment and capture between 1 to 96 library pools, allowing users to pool libraries as either a 3-plex or a single-plex. The automated library and enrichment process of 96 samples/32 3-plex pools can prepare libraries for sequencing in approximately 12 hours total time with 45 minutes of hands-on time.

In comparison to the use of manual pipetting, the Illumina RNA Prep, (L) Tagmentation with Enrichment automated on the Biomek platform provides:

- Reduced hands-on time and increased throughput
- Reduction in potential pipetting errors
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Knowledgeable support

Spotlight

Biomek i7 Hybrid (Multichannel 96, Span-8)

System features deliver reliability and efficiency to increase user confidence and walk-away time

- 300 µL or 1200 µL Multichannel head with 1-300 µL and 1-1200 µL pipetting capability
- Span-8 pod with fixed and disposable tips
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with 45 positions
- Orbital Shakers, Peltiers, and Tip washing for controlling sample processing

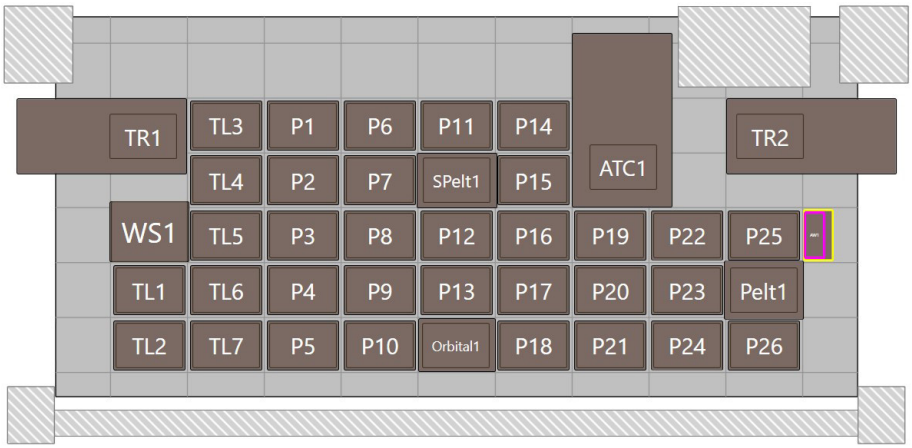

Figure 1. Biomek i7 Genomics Workstation. The layout shown above was used in the demonstration of the Illumina RNA Prep, (L) Tagmentation with Enrichment automated method.

Automated Method

The automated Illumina RNA Prep, (L) Tagmentation with Enrichment method is constructed in a modular fashion that follows the manual assay's recommended start and stop points, allowing the operator flexibility in performing the assay and allowing the automated method to be easily deployed in pre-PCR and post-PCR laboratory spaces. Enzymatic incubations and PCR reactions can be performed on-deck with an integrated thermocycler or with an off-deck thermocycler. Target capture requires the use of the shaking Peltier to heat the wash buffers used for washing the captured library pools.

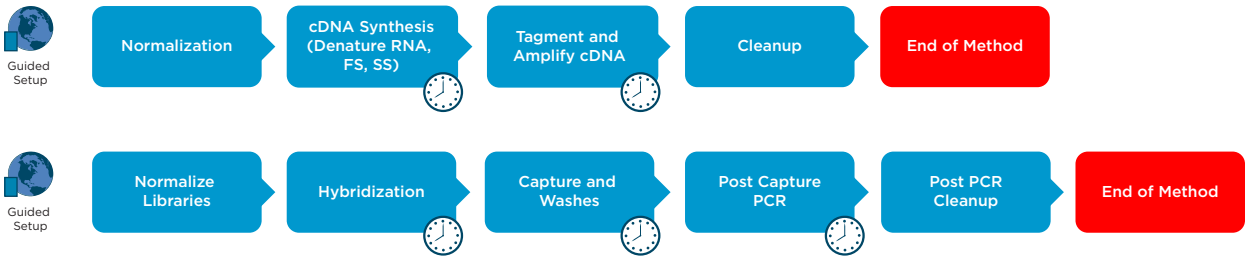

Figure 2. Illumina RNA Prep, (L) Tagmentation with Enrichment automated method workflow.

Automation provides increased efficiency by reducing hands-on time. In the table below, we detail the estimated time to complete the two workflows of the automated method with different sample number inputs.

cDNA Synthesis and Library Construction	32 Libraries	96 Libraries
Prepare Reagents/Set Up Biomek	15 min	30 min
Method Run	5 hr, 32 min	6 hr, 5 min
Total	**5 hr, 47 min**	**6 hr, 35 min**

Library Pooling and Enrichment	96 Libraries to 32 3-plex Pools	96 Libraries to 96 1-plex Pools
Prepare Reagents/Set Up Biomek	15 min	30 min
Method Run	5 hr, 16 min	5 hr, 58 min
Total	**5 hr, 31 min**	**6 hr, 28 min**

Table 1. Estimated run times for processing 32 and 96 libraries in library construction and 32 and 96 library pools in target enrichment. Timing estimates for method run times were obtained from Biomek software. Timing estimates include incubations and thermocycling, but do not include sample preparation or reagent thawing. Hybridization incubation time was estimated at minimum required time.

The software provides several user-friendly features such as

1. Biomek Method Launcher (BML)

BML is a secure interface for method implementation without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interact with the instrument if needed. The automated method may also be run through the standard Biomek software if Biomek Method Launcher is unavailable.

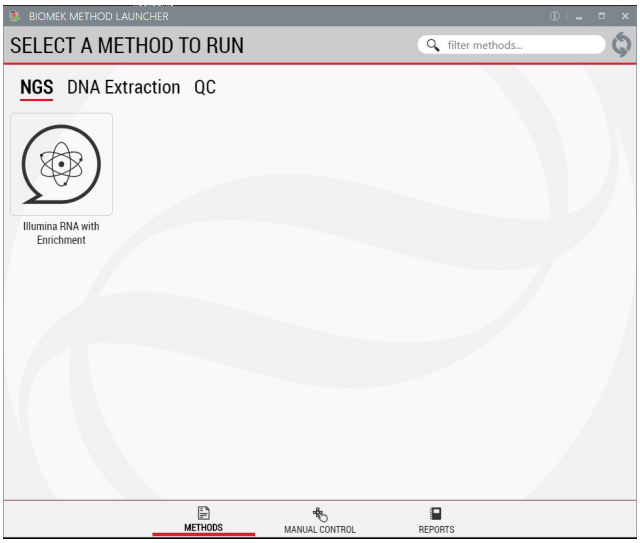

Figure 3. Biomek Method Launcher provides an easy interface to launch the method.

2. Method Options Selector (MOS)

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

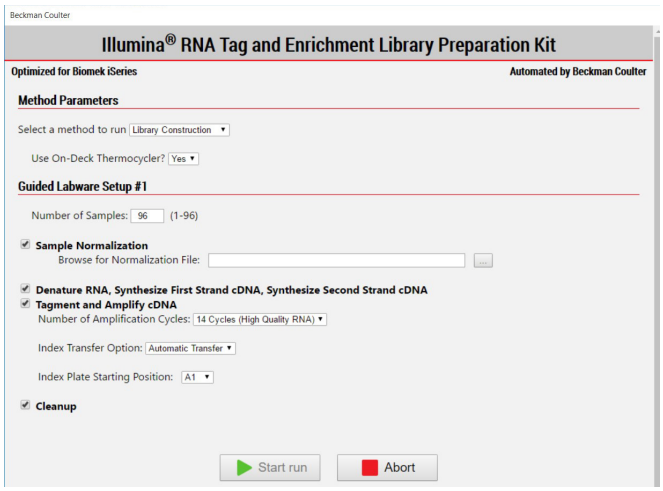

Figure 4. Biomek Method Option Selector enables users to select the desired workflow, sample number, and a variety of workflow customization options.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS and provides the user specific graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents. DeckOptix Final Check (part of BML) utilizes the Biomek's built-in camera system to verify labware placement to provide another layer of protection against incorrect instrument setup.

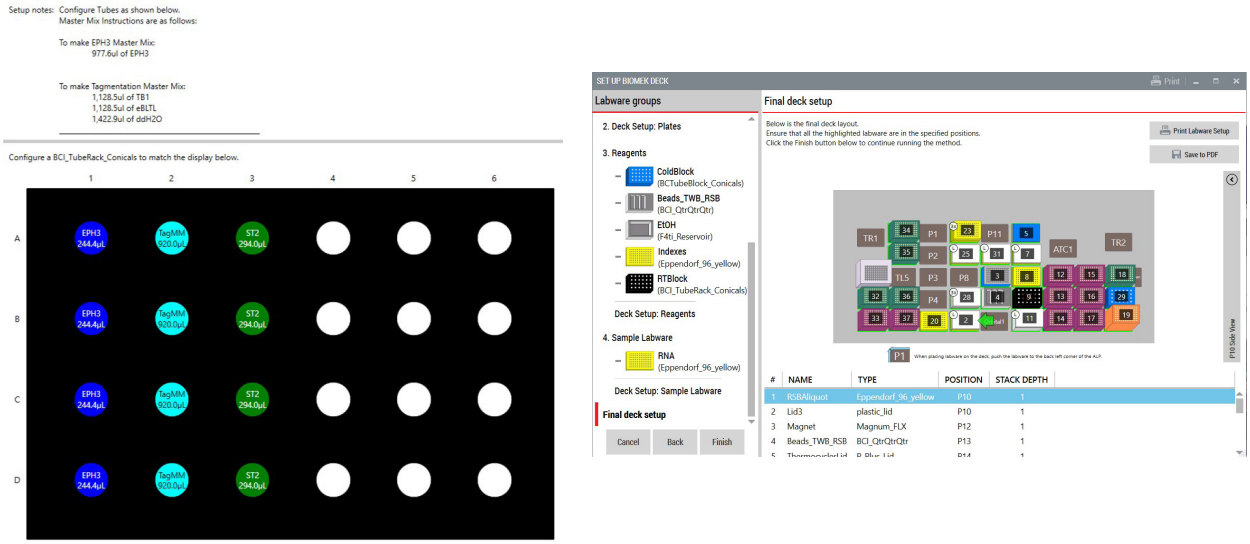

Figure 5. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

Experimental Design

Clinical patient samples that tested positive for SARS-CoV-2 were collected, purified, and de-identified by Berlin Institute of Health, Berlin, DEU. Two batches of 48 de-identified clinical samples were used to prepare Illumina RNA Prep libraries and enriched using the Respiratory Virus Oligo Panel v2 (Illumina). Enriched libraries were sequenced on an Illumina NextSeq using a mid-output NextSeq 500 reagent kit. Sequencing data was analyzed using the DRAGEN RNA Pathogen Detection App on Illumina BaseSpace and the Berlin Institute of Health Core Unit Genomics pipeline.

Results

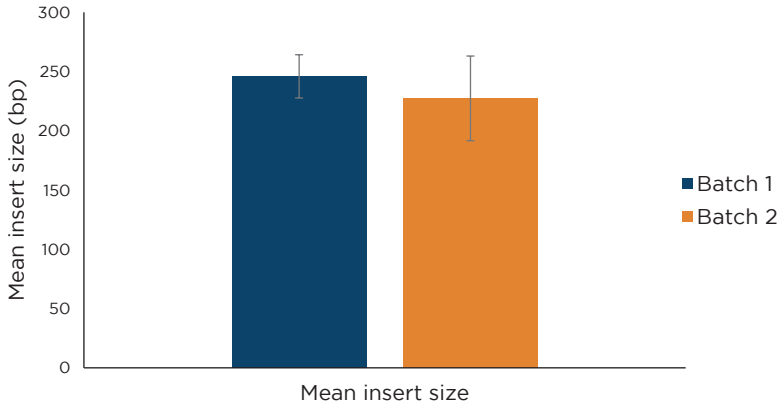

Figure 6. RNA Prep, (L) Tagmentation with Enrichment libraries mean insert size (bp) between two de-identified clinical batches. Error bars indicate standard deviation of the sample set.

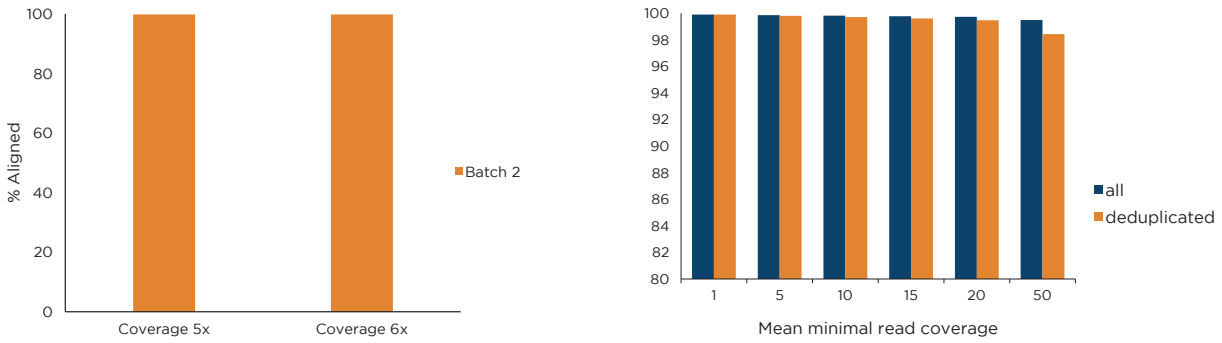

Figure 7. SARS-CoV-2 genome sequencing coverage of two de-identified clinical batches using the Illumina RNA Prep, (L) Tagmentation with Enrichment kit. Deduplicated reads are remaining reads after removal of PCR-duplicates.

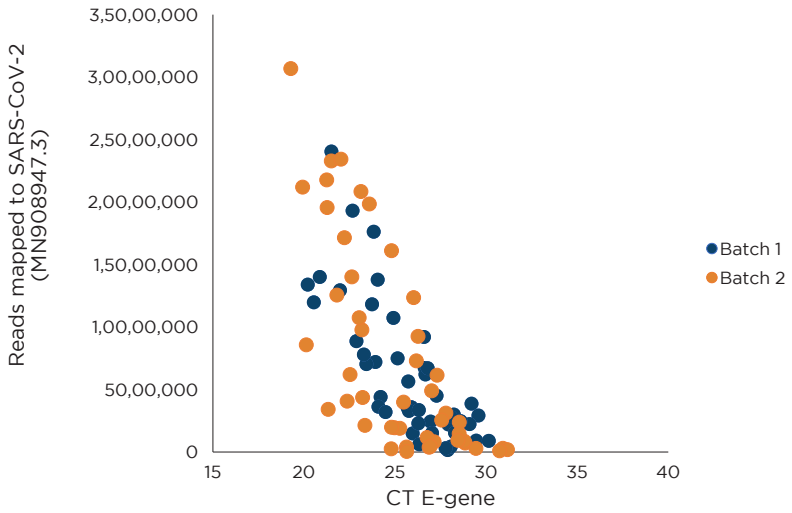

Figure 8. SARS-CoV-2 detection rates correlate with patient viral load across two different sample batches. CT values from RT-PCR of de-identified clinical samples were plotted against the number of reads that mapped to SARS-CoV-2 after analysis of sequencing results in Illumina BaseSpace using the DRAGEN RNA Pathogen Detection App and custom BIH Genomics Core Unit pipeline.

Conclusion

We have demonstrated that automation of the Illumina RNA Prep, (L) Tagmentation and Enrichment assay on the Biomek i7 Hybrid Genomics Workstation delivers high-quality libraries that enables high-sensitivity COVID-19 genome sequencing. By providing a robust automation solution for this assay, we empower researchers to take full advantage of Illumina's high-output sequencing technology.

Acknowledgements

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The Illumina RNA Prep, (L) Tagmentation with Enrichment kit is for Research Use Only. The Illumina RNA Prep (L) Tagmentation with Enrichment 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 labeled for IVD use and are not intended or validated for use in the diagnosis of disease or other conditions.

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