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Case study: Leveraging acoustic and tip-based liquid handling to increase throughput of SARS-CoV-2 genome sequencing

"Combining the Beckman Echo acoustic liquid handler and the Biomek i7 hybrid tip-based liquid handler has allowed us to greatly increase SARS-CoV-2 sequencing throughput."

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Introduction

Genomic surveillance of the SARS-CoV-2 virus is critical to pandemic response for two reasons. First, in order to detect variants of concern, it is estimated that a minimum of 5% of positive samples must be sequenced. Globally, this can mean tens of thousands of samples per day. Second, if a variant arises that escapes vaccine-mediated immunity, early detection will be critical to preventing the spread of these potentially disruptive variants. In this case study, we demonstrate how Echo acoustic liquid handling combined with Biomek tip-based liquid handling and amplicon-only sample preparation methods can greatly increase the throughput and lower tip usage for SARS-CoV-2 sequencing.

Workflow

We used a modified tailed ARTIC SARS-CoV-2 library prep method based on a tailed ARTIC SARS-CoV-2 method from NEB and a panel that generates amplicons of 275bp instead of the more standard 400bp[1,2]. Total nucleic acid or viral RNA fragments were extracted from positive SARS-CoV-2 specimens. 8 µL RNA from a source plate can be transferred into Echo LDV plates containing 2 µL NEB Lunascript RT SuperMix (Figure 1). After reverse transcription, the Echo 525 acoustic liquid handler transferred 1 µL of cDNA into a PCR plate containing Q5 Hotstart Master Mix and tailed TruSeq ARTIC primers for pool 1 or pool 2. After PCR, the two pools were combined and diluted 1:100 using Biomek i7 hybrid workstation. Next, 1 µL of the diluted cDNA was barcoded with unique dual indices in a PCR reaction using NEB Q5 Ultra II Master mix. After the indexing PCR, the 384 multichannel pod of the Biomek i7 hybrid transferred the products into an Echo LDV plate. The Echo was then used to pool an equal volume of each sample. Lastly, a single SPRI bead clean-up was performed on the pool instead of 384 individual cleanups. The resulting 275 bp amplicons tiling the viral genome were sequenced on a PE150 Illumina NovaSeq run. With this new protocol[3], each sample used three tips and the time from purified RNA to library is under 8 hours for 384 samples.
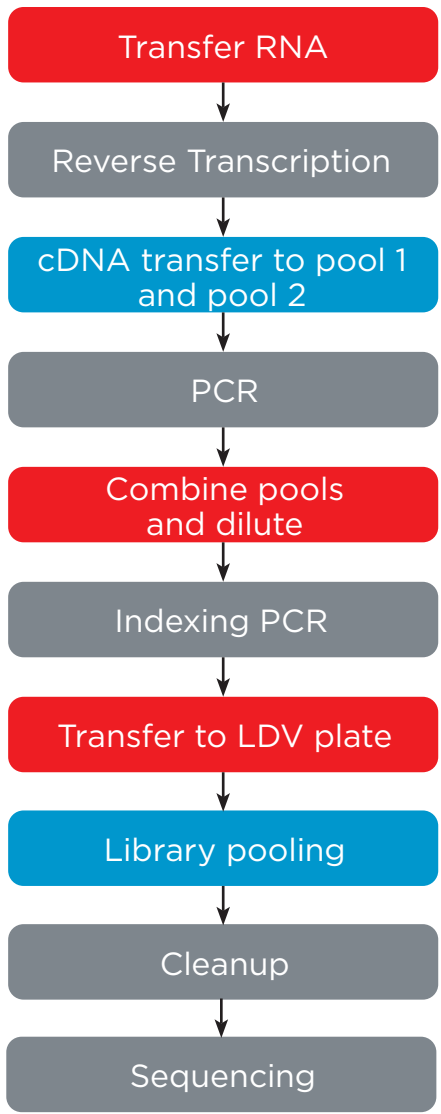
Figure 1. Sample preparation workflow utilizing Biomek i7 hybrid and Echo 525 liquid handlers. Biomek steps are indicated in red while Echo steps are denoted by blue.

Data

We used an Illumina NovaSeq6000 to run 384 barcoded libraries per lane of an SP 300 flow cell for at least 1 million reads per sample. The data below is a representative coverage plot of samples analyzed with IDSeq[4]. These samples have average viral C_t's of 15.6 (Figure 2 (a)) and 30.3 (Figure 2 (b)) and were prepared with our new 275bp tailed ARTIC SARS-CoV-2 approach, which shows even coverage and greater than 92% of genome called.
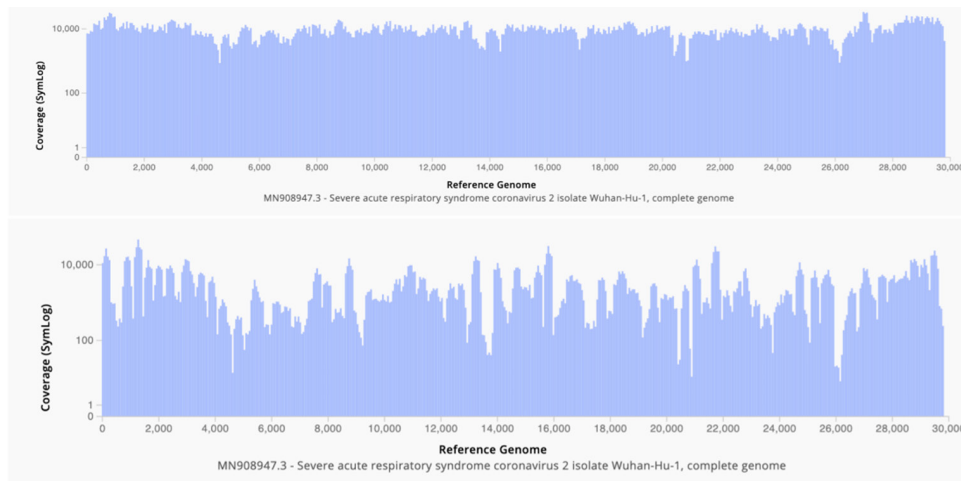

Figure 2. Genome coverage of the libraries prepared with the modified tailed ARTIC SARS-CoV-2 library prep workflow. The figure shows the coverage of samples with average viral C_t of 15.6 (a) and 30.3 (b)

Summary

Utilizing both the Echo acoustic liquid handler and the tip-based Biomek i7 Hybrid liquid handler with this new protocol enables our group to process several 384-well plates of SARS-CoV-2 samples per day, greatly increasing our throughput. The additional benefit of using Echo source plates is the reduction in pipet tips which are currently in short supply across the industry.

Highlights

This new 275bp tailed ARTIC SARS-CoV-2 protocol allows samples to be run on any Illumina platform by removing the requirement for PE250 sequencing.

- High throughput sample preparation: Combined use of the Biomek i7 hybrid and Echo 525 liquid handlers allows for an efficient, accurate, accelerated workflow which significantly increases output.
- Minimize tip usage: Use of the Echo 525 makes it possible to complete the library preparation using 3 tips per sample.
- Faster turnaround: Sample preparation can be completed in under 8 hours with a single bead clean up and faster sequencing with PE150 sequencing runs.

Acknowledgment

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References

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