In December 2019, an unknown emerging pathogen that causes severe acute respiratory syndrome spread to Wuhan, China. By mid-January 2020, there were at least 41 confirmed cases and one death. On January 12th, the genome sequence of this pathogen was published, and it was identified as a novel type of coronavirus (SARS-CoV-2) that is related to SARS-CoV and MERS-CoV. Since December, this RNA virus has spread to the rest of the world. Currently, in the U.S. alone, there are nearly 25 million confirmed cases and over 400,000 deaths. Thus, developing fast, reliable, and accurate methods for SARS-CoV-2 sequencing has become a worldwide necessity.

The current and most widely used protocol for SARS-CoV-2 library preparation is based on the ARTIC Network’s nanopore sequencing protocols for real-time detection of viral outbreaks, such as Ebola and influenza. As SARS-CoV-2 infections spread, the ARTIC Network nCoV2019 (i.e., SARS-CoV-2) sequencing protocol was quickly developed and updated. NextGen sequencing technologies have been invaluable in helping to identify, confirm, monitor, and trace SARS-CoV-2 mutations. Among these NextGen sequencing tools, Oxford Nanopore Technology (ONT) has one of the most widely accepted platforms. This is due to ONT’s remarkable portability, ease of operation, and fast turnaround time. Here we report that we have optimized the ARTIC Network SARS-CoV-2 sequencing protocol for ONT platforms by simplifying steps, reducing costs, and improving amplicon coverage.

With this approach, we have assembled an all-in-one kit (NEBNext® ARTIC SARS-CoV-2 Companion Kit) that can be used in conjunction with the ONT Native Barcoding Kit/Ligation Sequencing Kit to generate targeted amplicon libraries for MinION, GridION, and other ONT sequencing platforms. This all-in-one kit demonstrates improved SARS-CoV-2 genome coverage and variant calling. The kit can also be easily adapted for use with current and previous ARTIC Network SARS-CoV-2 sequencing protocols, as well as the PCR filing of a virus culture from ONT. Furthermore, this kit can be modified by swapping out our primer pools with any other amplicon-specific primer sets to sequence any other known viral genomes.

Methods

**Figure 1.** Workflow based on ARTIC network nCoV2019 sequencing protocol for preparing cDNA sequencing library on ONT platforms. cDNA from SARS-CoV-2 RNA is prepared by reverse transcription and PCR amplification using balanced v3 nCoV2019 PrimerSeq sequencing Primers. Pools of overlapping tiled amplicons are generated by two PCR reactions using two different set of primers, 30 ng of the amplified amplicons are end-paired and dA tailed followed by barcode ligation. Then, barcoded samples are pooled and cleaned by beads followed by ligation to the Nanopore sequencing adapter using 5ng from each barcoded sample. 6-24 samples can be processed and pooled for a single sequencing run on MinION or GridION.

**Figure 2.** Components in the kit

**Figure 3.** Time to prepare the cDNA sequencing library

**Figure 4.** Reduced SARS-CoV-2 RT-PCR Amplification

**Figure 5.** Improved SARS-CoV-2 Genome Coverage with NEBNext Protocol and Reagents for Nanopore Sequencing

**Figure 6.** Fraction of Genome Covered

**Figure 7.** Adequate coverage of the SARS-CoV-2 genome for both NEB primers and MidPrim primers

**Figure 8.** NEBNext ARTIC SARS-CoV-2 kit is compatible with nCoV-2019 sequencing protocol v3 (LoCost)

Conclusions

- NEBNext ARTIC SARS-CoV-2 Companion Kits have a streamlined protocol and minimized costs by the reduction of reaction volumes
- The primer set in the NEBNext ARTIC SARS-CoV-2 kit has been balanced to achieve an improved genome coverage for SARS-CoV-2 genome comparing to v3 commercially available primer set
- Protocol has been optimized to have the best compatibility with nCoV-2019 sequencing protocol v3 LoCost and Nanopore PCR filing of COVID-19 virus protocols