

# **An Improved Multiplex Targeted Amplicon Sequencing of SARS-CoV-2 Using Oxford Nanopore Technology**

## **Genomics**

**Luo Sun** (sun@neb.com), New England Biolabs

In December 2019, an unknown emerging pathogen that causes severe acute respiratory syndrome started to spread in 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 30 million confirmed cases and 500,000 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 nCoV-2019 (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 been 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-CoV2 genome coverage and variant calling. The kit can also be easily adopted for use with current and previous ARTIC Network nCov-2019 sequencing protocol versions, as well as the PCR tiling of COVID-19 virus protocol 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.