Improving multiplex targeted amplicon sequencing of SARS-CoV-2 on Illumina platforms.

Core Administration

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The impact of the novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) on the global community has produced a critical need to develop reliable and accurate protocols for sequencing emergent pathogens. In response to this critical need, NEB applied our molecular expertise and resources to expand and improve upon ARTIC-based SARS-CoV-2 sequencing methods. The initial ARTIC SARS-CoV-2 sequencing protocol published in January of 2020 (Josh Quick 2020) is based on a multiplexed amplicon whole-viral-genome Oxford Nanopore Technologies[®] sequencing approach. Here we present refined protocols and reagents for the sequencing of SARS-CoV-2 on Illumina[®] sequencing platforms.

The methodology demonstrated here incorporates optimized DNA polymerase master mixes that promote robust and high-throughput multiplex targeted amplicon sequencing on Illumina platforms. NEBNext® ARTIC SARS-CoV-2 kits contain all reagents needed for RT-PCR and downstream library preparation. NEBNext ARTIC reagents for RT-PCR deliver ample amplicon yields from genomic RNA across a wide copy-number range. Furthermore, we have eliminated the need to normalize amplicon concentration prior to library prep by employing a novel DNA polymerase formulation for library enrichment.

With these innovative reagents and techniques, we have developed two methods for SARS-CoV-2 sequencing on Illumina platforms. One follows a whole-amplicon sequencing approach, while the other utilizes enzymatic fragmentation and allows for sequencing on a 150-cycle MiSeq[®] flow cell. The same cost-effective RT-PCR protocol is used prior to library prep for both approaches. The streamlined NEBNext ARTIC SARS-CoV-2 Companion Library Prep protocols exhibit consistent SARS-CoV-2 genome coverage. Ultimately, these enhanced protocols and reagents enable the accurate sequencing and tracking of SARS-CoV-2 viral strains across the globe. These methods can also be readily applied to diverse multiplexed amplicon sequencing tactics, such as the identification and tracking of novel pathogens in the future.