ABSTRACT

Amidst a pandemic caused by the SARS-CoV-2 virus, rapid genomic surveillance of the virus is much needed to support epidemiology monitoring of the pathogen. Oxford Nanopore Technologies (ONT) has allowed scalable and high-throughput SARS-CoV-2 sequencing. However, a considerable coverage drop in certain genome regions has been observed in experiments using the ARTIC V3 Classic Protocol (ARTIC V3 primer with SQK-LSK109) and Midnight Protocol (Midnight primer and SQK-RBK110.96). This study aims to optimize the annealing temperature of the primer set and evaluate the performance of each SQK-LSK109 and SQK-RBK109 library preparation kit, as well as the protocol, respectively. Three Delta variant samples with a Ct value of 18, 23, and 25 are used in this study. Gradient PCR tiling was conducted before the sequencing to determine the optimized annealing temperature. The sequencing was done in two batches; batch one using SQK-LSK109 and batch two using SQK-RBK110.96, each with the sample amplified using ARTIC V3 and Midnight primer, respectively. Out of five temperatures, only three (59°C, 61°C, and 63°C) yielded the most consistent and greater viral genome coverage with minimal amplicon drops. While there are no remarkable differences in the sequencing quality of both library preparation kits regardless of the primer, the Midnight protocol demonstrates a greater genome recovery of the SARS-CoV-2 by >98% with fewer amplicon drops in optimized annealing conditions. The Midnight protocol with lower optimized annealing temperature allows rapid and high-quality genomic sequencing to support epidemiology surveillance.

Keywords: SARS-CoV-2, ARTIC V3 primer, Midnight primer, SQK-LSK109 kit, SQK-RBK110.96 kit.

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This report is far from perfect and still has room for improvement. Hence, constructive feedback and input are always welcomed. It is my sincere hope that this study will be beneficial, especially for clinical laboratories that are still fighting against SARS-CoV-2.

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