A SISPA-nanopore Sequencing Approach for Genomic Characterization of Viruses from Clinical Samples

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Abstract

Background: Genomic characterization of viruses directly from clinical samples remains technically challenging because of low viral load, high host nucleic acid background, and incomplete recovery of viral genetic material. These limitations can affect nucleic acid quality and viral nucleic acid recovery, thereby reducing library quality and sequencing performance. Although Oxford Nanopore sequencing provides rapid and flexible sequencing capability, successful characterization from clinical samples still depends on sufficient recovery of viral nucleic acids. While direct RNA sequencing is now available, its routine application in diagnostic and surveillance settings may still be limited by relatively high cost and practical workflow considerations. Sequence-Independent Single-Primer Amplification (SISPA) is therefore a useful amplification-based approach for enhancing viral sequence recovery prior to sequencing and supporting direct genome-based characterization of dsRNA viruses from clinical samples.

Objectives: This study aimed to evaluate a SISPA-based Nanopore sequencing workflow for genomic characterization of dsRNA viruses directly from clinical samples and compare sequencing outcomes generated from three sample preparation workflows—cDNA synthesis, double-stranded cDNA synthesis, and SISPA—Representative dsRNA viruses, including Bluetongue virus (BTV) and African horse sickness virus (AHSV), were used as target viruses in this study.

Methods: Viral RNA was extracted from clinical samples using the QIAamp Viral RNA Mini Kit. The extracted RNA was processed using three separate preparation workflows for comparative analysis: cDNA synthesis using random hexamers, double-stranded cDNA synthesis, and sequence-independent single-primer amplification (SISPA). Libraries generated from each workflow were prepared using a Native Barcoding Kit and sequenced on an Oxford Nanopore MinION Mk1C platform with an R10 flow cell. Basecalling was performed using Dorado v7.6.7 integrated in MinKNOW v24.11.8. Reads were adapter-trimmed using Porechop v0.2.4, quality-assessed using NanoPlot v1.43.0, and length-filtered using Chopper v0.8.0. Reference-based analysis was conducted using minimap2 v2.28, SAMtools and BCFtools v1.21 for read mapping, sorted alignments and consensus sequences. Viral detection was defined as the presence of mapped viral reads against the reference genome, while successful sequence recovery was determined based on ≥50% genome coverage with a mean sequencing depth of ≥30× in reference-based analysis.

Results: Comparative analysis showed that the SISPA workflow yielded the most informative sequencing output among the three preparation methods. Relative to cDNA and double-stranded cDNA, the SISPA workflow markedly improved viral read recovery, with approximately 518.5-fold (1,037 vs 2 reads) and 148.1-fold (1,037 vs 7 reads) higher viral read counts, respectively. Although limited numbers of mapped viral reads were identified in the cDNA and double-stranded cDNA datasets, these workflows did not meet the predefined criteria for successful sequence recovery. In contrast, the SISPA-derived dataset successfully recovered the target viral sequence, achieving the defined genome coverage and sequencing depth thresholds in reference-based analysis.

Conclusion: These findings indicate that SISPA can enhance Nanopore-based recovery of dsRNA viral sequence data from a clinical sample and enable downstream reference-based analysis. This workflow provides a useful approach for the genomic characterization of dsRNA viruses directly from clinical samples.

Keywords: Sequence-Independent Single-Primer Amplification (SISPA), Oxford nanopore sequencing, Clinical samples, RNA viruses, Viral sequencing

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