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

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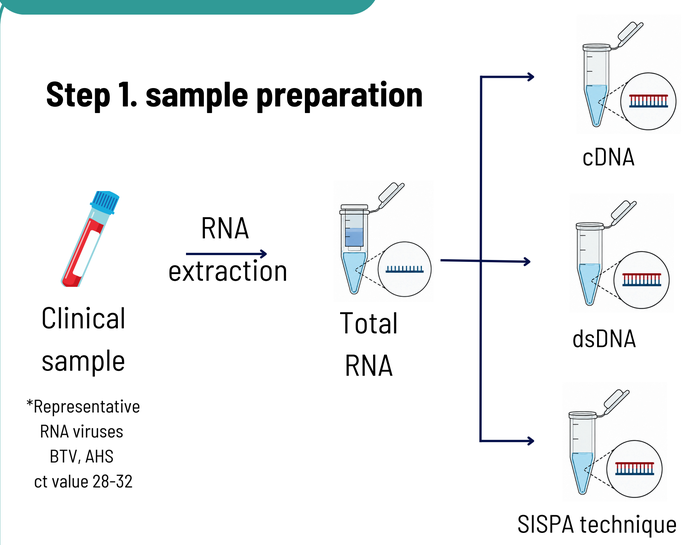
Background

Direct viral genome sequencing from clinical samples can be limited by low viral abundance and high host nucleic acid background, resulting in insufficient recovery of viral genetic material for downstream genomic characterization. Sequence-Independent Single-Primer Amplification (SISPA) has been proposed as a pre-sequencing enrichment strategy to improve viral nucleic acid recovery. Therefore, this study compared cDNA, ds-cDNA, and SISPA workflows for Oxford Nanopore-based genomic characterization of RNA viruses from clinical samples.

Methods



ACKNOWLEDGEMENTS

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References

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2. Reyes GR, Kim JP. Sequence-independent, single-primer amplification (SISPA) of complex DNA populations. Mol Cell Probes. 1991 Dec;5(6):473-81. doi: 10.1016/s0890-8508(05)80020-9

Results and Discussion

Figure 1. Comparison of viral reads recovery among sample preparation workflows

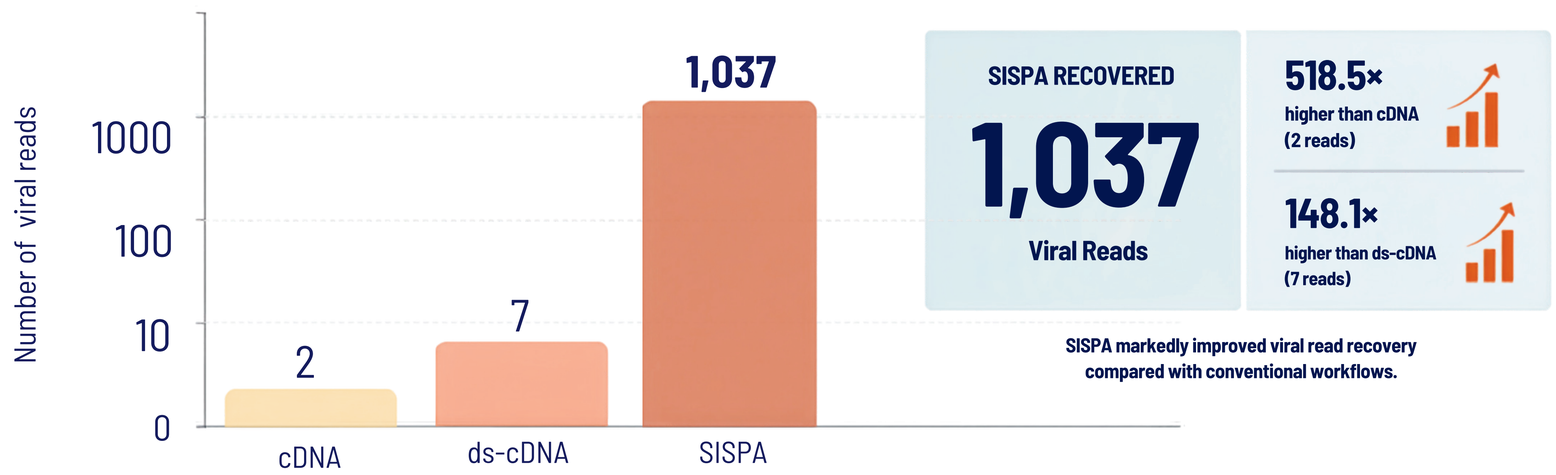


Figure 2. Genome coverage and sequencing dept of SISPA workflow

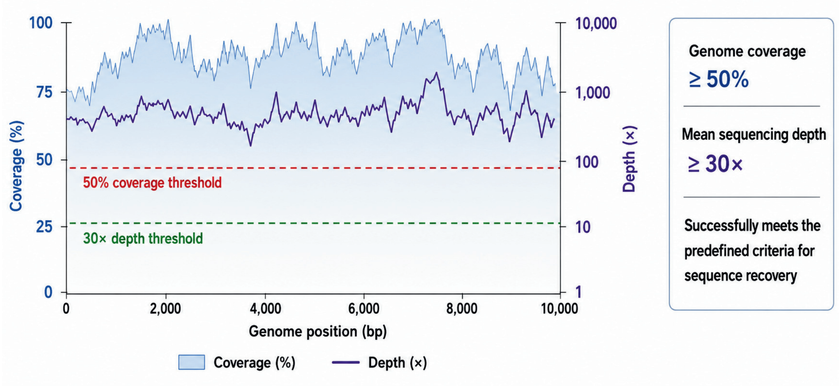
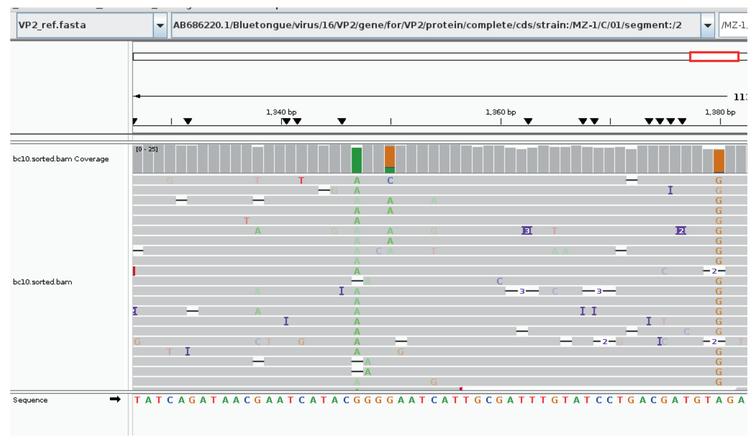


Figure 3. Representative IGV alignment of SISPA-derived reads mapped to the VP2 gene of the BTV reference genome.



As shown in Figure 1, the SISPA workflow generated substantially higher viral read recovery (1,037 mapped viral reads) than the cDNA and ds-cDNA workflows, which yielded only 2 and 7 mapped viral reads, respectively. The marked increase in viral read recovery suggests that SISPA improved the representation of viral nucleic acids in the sequencing library, thereby enhancing the ability to recover viral genomic information from clinical samples.

Furthermore, Figure 2 demonstrates that the SISPA workflow achieved genome coverage greater than 50% and a mean sequencing depth exceeding 30×, meeting the predefined criteria for successful sequence recovery. In contrast, the limited number of viral reads obtained from the cDNA and ds-cDNA workflows was insufficient to achieve comparable genome coverage and sequencing depth, restricting downstream genomic characterization.

The representative IGV alignment shown in Figure 3 further supports the successful recovery of viral genomic sequences obtained using the SISPA workflow. These findings suggest that amplification-based enrichment prior to sequencing can substantially influence viral sequence recovery and downstream genomic analysis from clinical samples.

Conclusions

SISPA improved viral sequence recovery compared with the cDNA and ds-cDNA workflows and enabled successful downstream genomic characterization. These findings support the use of SISPA as a pre-sequencing enrichment strategy for Nanopore-based analysis of RNA viruses directly from clinical samples.