

HIFIVIRAL SARS-COV-2 KIT FOR COVID-19 WHOLE GENOME SEQUENCING – BEST PRACTICES

HiFiViral for SARS-CoV-2 is a simple-to-use, scalable, cost-effective solution for sequencing the entire SARS-CoV-2 genome. This fully kitted solution uses a novel approach that is robust to new variants and comprehensively detects all types of mutations.

Why choose HiFiViral for SARS-CoV-2?



Robust performance: Our fully kitted end-to-end solution uses a molecular inversion probe (MIP)-based method that combines the simplicity and cost effectiveness of PCR with the robustness of target capture methods. Each base is covered by 22 probes, making the assay resilient to viral SNVs and indels.



Capture all variants: The combination of 675 bp targets and the accuracy of HiFi sequencing enables comprehensive detection of both SNVs, indels, and structural variants.

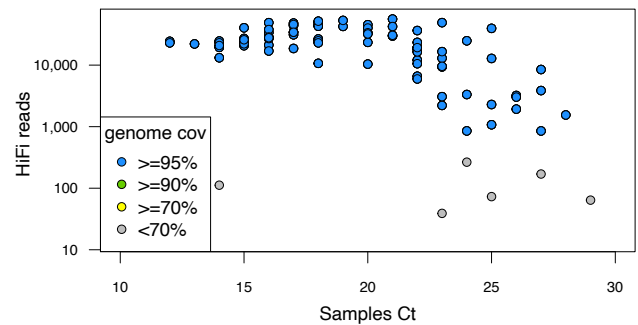


Scalability: HiFiViral for SARS-CoV-2 provides cost-effective, built-in surge capacity to scale throughput on a single platform.



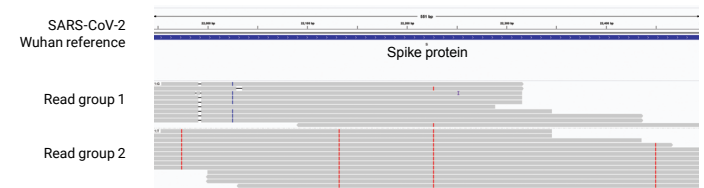
Simple to use: The PacBio HiFiViral kit requires up to 80% less labor than PCR-based enrichment assays. The add-only enrichment workflow further reduces handling errors with premixed reagents and color-change indicators at every step.

Robust performance on nasopharyngeal swabs



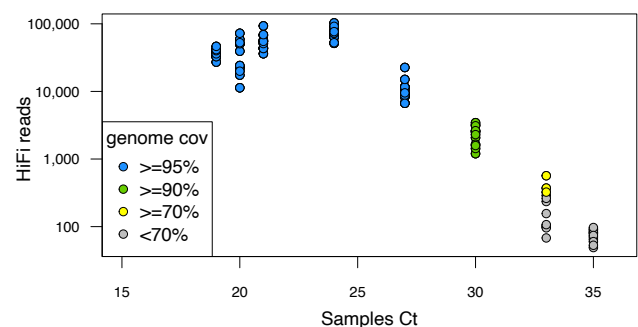
RNA extracted from nasopharyngeal swabs with a broad range of Ct values was processed with the HiFiViral SARS-CoV-2 kit, pooled at 96-plex, and sequenced on a Sequel[®] Ite system. Overall, 93% of samples yielded genome coverage $\geq 90\%$.^{1,2}

Accurate detection of all mutation types



The HiFiViral SARS-CoV-2 analysis application in SMRT[®] Link v10.2 provides sample consensus sequences, calls mutations against a reference, and flags potential multi-strain samples. In this surveillance sample, reads mapped to the S gene show evidence of two co-occurring strains.^{1,2}

Consistent results with commercial control samples



Control samples were diluted to mimic samples with Ct values from 20 to 35 and sequenced at 96-plex. Samples with Ct ≤ 30 yielded complete genomes with as few as 1,000 mapped HiFi reads.^{1,2}

HIFIVIRAL FOR SARS-COV-2 WORKFLOW RECOMMENDATIONS



Enrich and barcode samples for SARS-CoV-2 RNA³

- Start with RNA extracted from up to 384 samples
- Pool enriched samples and generate a SMRTbell[®] library
- Complete viral enrichment with < one hour hands-on time

Add extracted RNA samples to the assay plate.
The remaining enrichment workflow is add-only.



1. Add **RT reagent + probe mix**



2. Add **fill-In mix** to create circular DNA



3. Add **cleanup mix** to remove non-circular RNA/DNA



4. Add PCR mix and premixed primers to **barcode + amplify**



5. Pool samples and proceed to SMRTbell library prep



Sequence samples on the Sequel II or Sequel IiE system

- Load up to 384 samples on one SMRT[®] Cell 8M
- Load up to 8 SMRT Cells with one touch point to process 3,072 samples per week



Analyze data with SMRT Link v10.2

- Overnight sequencing and analysis with one touch point
- Generate submission-ready files and troubleshoot with intuitive QC tools
- Go from viral RNA to answers in 28 to 42 hours

KEY REFERENCES

1. Presentation: Kingan, S. (2021) **HiFiViral: A novel method for surveillance of SARS-CoV-2 that is robust across sample input quantities and the evolution of new variants.** American Society of Human Genetics Virtual meeting 2021.
2. Poster: Kingan, S., et al. (2021) **HiFiViral SARS-CoV-2: A kitted solution for genome surveillance that is robust across sample input quantities and new variants.** SFAF 2021 poster.
3. **Procedure + checklist – PacBio HiFiViral high-throughput multiplexing for full-viral genome sequencing of SARS-CoV-2.** PacBio documentation.



Learn more about the HiFiViral SARS-CoV-2 kit for COVID-19: pacb.com/COVID-19

Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at <http://www.pacb.com/legal-and-trademarks/terms-and-conditions-of-sale/>. PacBio, the PacBio logo, SMRT, SMRTbell, and Sequel are trademarks of PacBio. All other trademarks are the sole property of their respective owners.

© 2022 PacBio. All rights reserved. For research use only. Not for use in diagnostic procedures.
102-193-692 REV02 19MAY2022

