



HiFiViral SARS-CoV-2: A kitted solution for genome surveillance that is robust across sample input quantities and new variants

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Introduction

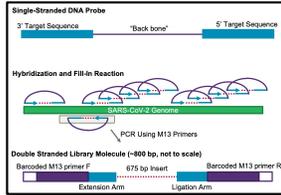
The COVID-19 pandemic continues to be a major global epidemiological challenge with the ongoing emergence of new strain lineages that are more contagious, more virulent, drug resistant and in some cases evade vaccine-induced immunity. In response, the HiFiViral SARS-CoV-2 kit (PacBio; Menlo Park, California) was developed as a scalable solution for the Sequel II and Sequel IIe systems.

HiFi Viral Uses Differentiated Enrichment Technology

Viral enrichment uses Molecular Inversion Probes (MIPs), single stranded DNA probes with high specificity and scalability.

Figure 1. Enrichment with Molecular Inversion Probes

- Probe has two complementary target sequences connected by a DNA backbone
- Fill-in reaction creates a circular molecule
- Universal primers add dual indices for sample multiplexing



Easy Workflow and Simple Kit Design

Figure 2. Workflow

- “Add-only” viral enrichment workflow (green)
- Hands on time of <1 hour for viral enrichment
- Overnight hybridization + 12 hour total prep time
- Sequencing and analysis in one overnight step
- Process up to 384 samples on one SMRT Cell 8M with Sequel II/IIe systems



Performance In Controls Across Input Quantities

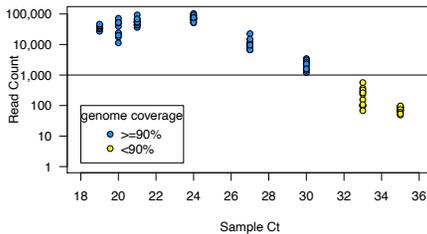
Table 1. Experimental Design: 96-plex prepared with 4 Synthetic RNA Controls at 8 input quantities in replicates of 3.

Twist Control	Variant	Part Number
14	Alpha (B.1.1.7)	103907
15	Alpha (B.1.1.7)	103909
16	Beta (B.1.351)	104043
17	Gamma (P.1)	104044

Sample Ct	Copy Number
19	6M
20	3M
21	1M
24	100,000
27	10,000
30	1,000
33	100
35	3

Table 2. Input Quantity
Input of RNA controls ranged from 6 million copies down to 3. Copy number is converted into Ct scale after Han et al. 2021.

Figure 3. Performance Across Range of Sample Ct Values (96-plex)



- 100% of samples with Ct < 32 have complete genomes (>90% genome covered).
- HiFi read depth of 4-fold or greater required to output consensus base.
- Samples with 1000 or more processed reads have complete genome coverage.

Variant Calling In Controls

- HiFi Reads were filtered on barcode quality and read length (<800bp)
- Barcoded primers and probe sequences were identified and trimmed
- Reads were mapped to Wuhan reference (NC_045512.2) with pbmm2 v1.7.0 and variants called with bcftools v1.13
- Consensus sequence generated with vcfcons
- Precision and recall were calculated for variant discovery against the Wuhan reference

Table 3. Variant Calling Accuracy

Twist Control	True Positive	False Positive	False Negative	Positive Predictive Value	Recall
14	35	0	0	1	1
15	31	0	0	1	1
16	24	0	0	1	1
17	34	0	0	1	1

Performance in Nasopharyngeal RNA Extracts

Data Collection: 96-plex prepared with 2 Synthetic RNA Controls, 1 NTC, 1 negative control, and 92 nasopharyngeal RNA extracts with Ct values between 10-30.

- 74 of 92 (80%) of nasopharyngeal extracts had complete genomes
- 88% of delta variant samples have complete genomes

Figure 4. HiFi Read Count Versus Sample Ct

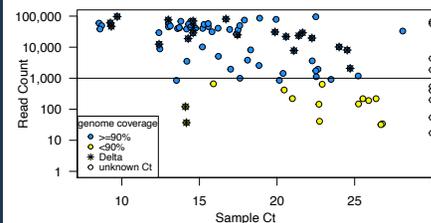
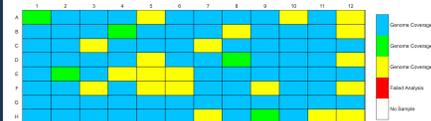


Figure 5. Example Assay QC Report



Genome Coverage

Figure 6. Genome Coverage In 96-plex

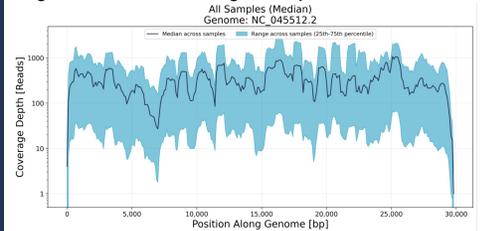


Figure 7. Coverage Across Spike Gene in Delta Sample

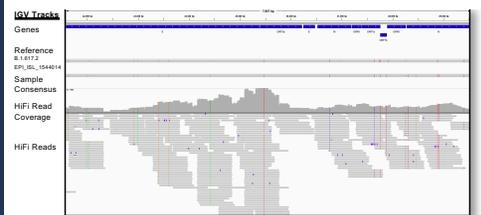


Table 4. Summary Stats for Delta Sample (Fig. 7)

Sample Ct	No. HiFi Reads	Median Read Depth	Genome Coverage	Pangolin Lineage
24.52	8071	179-fold	99.0%	B.1.617.2

Conclusion

The HiFiViral SARS-CoV-2 is an easy to perform solution for surveillance of variants to support pandemic response by public health. With 80% of samples yielding complete genome coverage in a 96-plex run, the combination of long read lengths and a differentiated probe design provides highly accurate results and robust genome coverage across a range of Ct values.

References and Acknowledgements

Han M.S., et al. (2021). RT-PCR for SARS-CoV-2: quantitative versus qualitative. *The Lancet Infectious Disease* 21(2) p165.
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