HiFiViral SARS-CoV-2: A mutation tolerant, fully-kitted solution for COVID-19 surveillance

Meredith Ashby[1], Yihe Wang[2], Sarah Hoffman[2], Frances Long[3], Nicole Madamba[3], Chirayu Goswami[2], Jason Ruggieri[2], Jessica Spangler[1], Caroline Storer[1], Chris Dunlay[3], Jonathan Schultz[2], Sarah Kingan[1]

1. PacBio, 1305 O'Brien Drive, Menlo Park, CA 94025; 2. Sampled, 30 Knightsbridge Road Bldg 3, Piscataway NJ 08854; 3. PerkinElmer, 68 Elm Street, Hopkinton MA 01748

Introduction

The COVID-19 pandemic is an ongoing global challenge, with the repeated emergence of new variants that are more contagious, more virulent, drug resistant or evade vaccine-induced immunity. In response, the HiFiViral SARS-CoV-2 kit was developed as a scalable solution with increased resilience against virus mutations, designed for use on the Sequel IIe system.

MIPs technology enables a simple workflow

Figure 1. The HiFiViral SARS-CoV-2 kit relies on ~1,000, densely tiled Molecular Inversion Probes (MIPs) such that every genomic position is covered by ~22 dual-specific probes, resulting in robust genome coverage of all circulating variants without the need for periodic primer updates

The workflow is simple to perform manually

- Addition-only viral enrichment workflow
- Color changes give a visual confirmation that each step has been performed correctly
- Hands-on time of <1 hr for viral enrichment
- Sequencing an analysis in one overnight step
- Process up to 384 samples in one SMRT Cell 8M with Sequel II/IIe Systems.

Performance in control samples

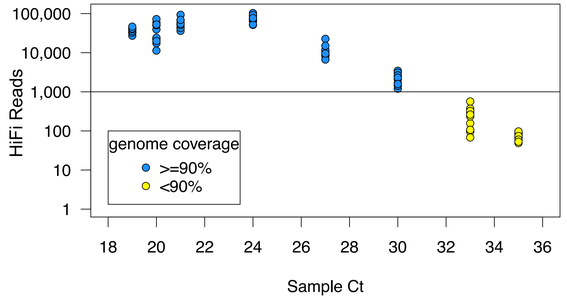
Table 1. Experimental design: 96-plex prepared with 4 synthetic RNA controls at 8 input quantities in replicates of 3.

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

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 2. 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 is required to output a consensus base
- Samples with 1000 or more processed reads have complete genome coverage.

Workflow automation

The HiFiViral workflow can be automated for 96 samples on the PerkinElmer Sciclone G3 NGSx workstation with user touch points for off-deck incubations and reagent plating.

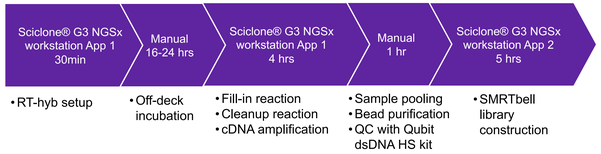
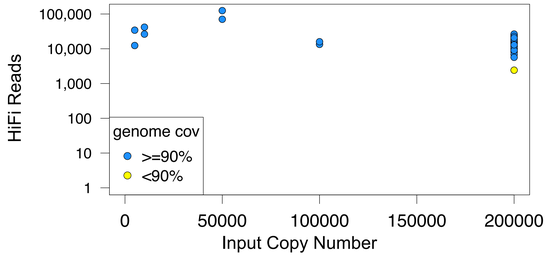


Figure 3. Performance with automated preparation. 91 out of 92 control samples have genome completeness > 95%.



Variant calling in controls

- 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 extracts

Figure 4. HiFiViral performance at 384-plex. Performance at high throughput was demonstrated on a combination of controls and nasopharyngeal (NP) extracts at 384-plex. 90% of the controls with Ct < 30 and 85% of the NP extracts had genome completeness > 90%.

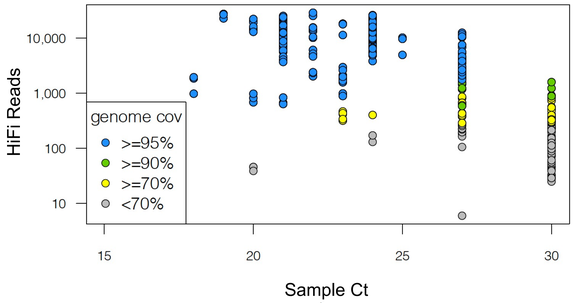
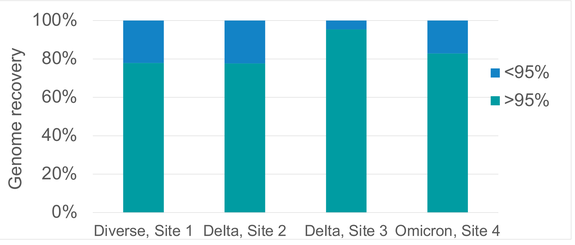


Figure 5. HiFiViral performance against widely circulating variants. Data from surveillance samples analyzed during the fall of 2021 and winter of 2022 at 4 distinct sites shows that performance remained consistent for runs comprised of samples from diverse lineages (Site 1, 95-plex), predominantly Delta (Site 2, 380-plex or Site 3, 87-plex), or predominantly Omicron (Site 4, 35-plex)



References

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