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

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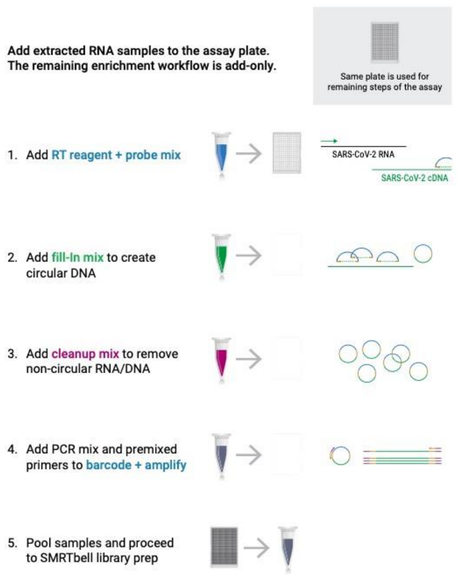
Introduction

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

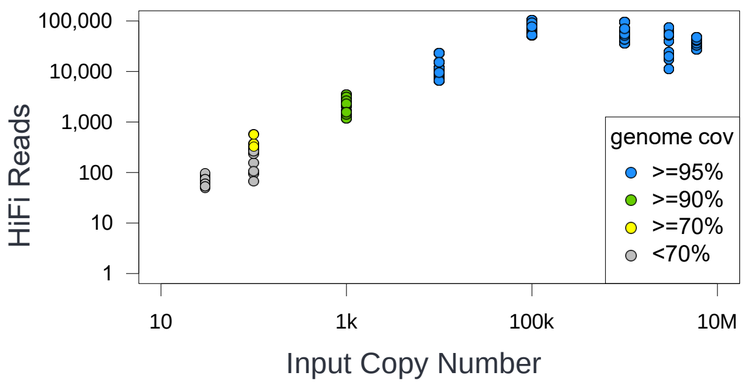
Figure 1. The HiFiViral SARS-CoV-2 kit relies on ~1,000, densely tiled Molecular Inversion Probes (MIPs) such that every genomic position is covered by ~22 dual-specific probes, resulting in robust genome coverage of all circulating variants without the need for periodic primer updates



- 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 and analysis in one overnight step
- Process up to 384 samples in one SMRT Cell 8M with Sequel II/IIe Systems.

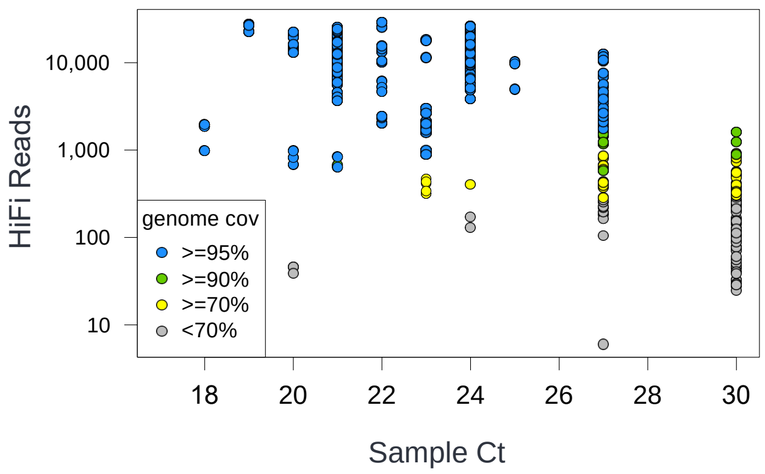
Sensitivity for input copy number in synthetic RNA controls

Figure 2. Performance at 96-plex. 90% genome coverage with 1000 copies; 95% genome coverage with minimum 10,000 copies. RNA controls are alpha, beta, and gamma lineages.



Performance in nasopharyngeal extracts

Figure 3. 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%.



Original probe design gives robust results for new variants

Figure 4. Genome completeness in sequencing runs for 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).

Table 1. Consensus sequence for emerging variants is high quality. 2483 sequences deposited into GenBank by the University of Louisville between 25 November 2021 and 25 April 2022 were analyzed in NextClade.

Clade	Number Samples	Percent "good" quality	Percent with <250 missing bases	Percent with 0 missing bases
21A (Delta)	17	76%	88%	88%
21I (Delta)	108	94%	85%	72%
21J (Delta)	2095	90%	80%	70%
21K (Omicron)	242	93%	85%	55%
21L (Omicron)	14	100%	100%	64%

Automation with PerkinElmer Sciclone G3 NGSx workstation

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.

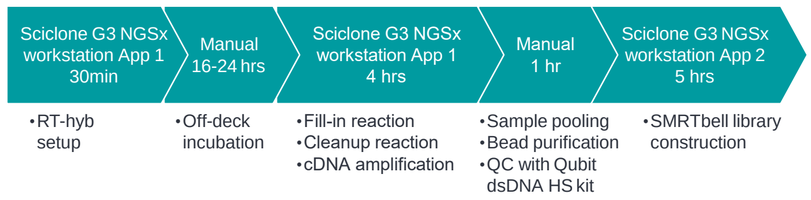
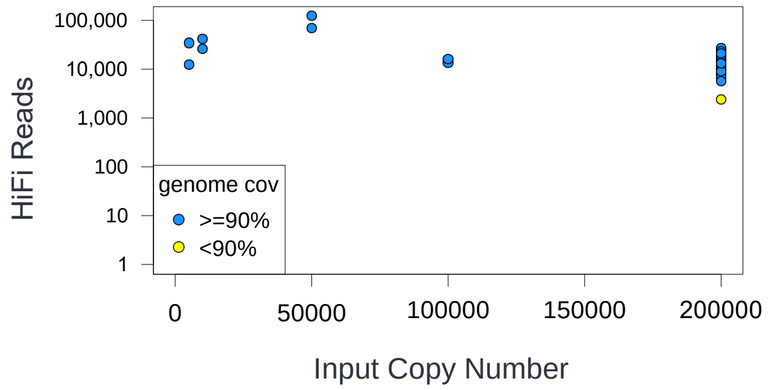


Figure 5. Performance with automated preparation in controls. 91 out of 92 control samples have genome completeness > 95%.



High throughput (HT) automation solution

Manual set-up at 384-plex is not possible due to time and temperature sensitivity of hybridization workflow. The PerkinElmer Zephyr G3 NGS Workstation is used to plate RNA extracts and the SPT Labtech mosquito HV Genomics is used to transfer reagents with a 16-channel head. User touch points needed for off-deck incubation and reagent plating.

mosquito HV Genomics from SPT Labtech

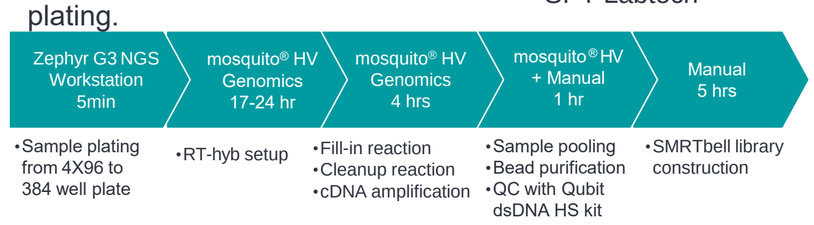


Figure 6. Performance in surveillance samples with HT automation. 191 of 372 surveillance samples have genome completeness greater than 90%.