APPLICATION NOTE

PacBio HiFiViral SARS-CoV-2 Kit automated on Sciclone G3 NGSx workstation.

Introduction

The COVID-19 pandemic is an ongoing global challenge with new variants continuing to emerge. The PacBio® HiFiViral™ SARS-CoV-2 kit was developed as a scalable solution with increased resilience against virus mutations, designed for use on the PacBio® Sequel lle system. The kit was automated on the Sciclone G3 NGSx workstation to enable a high-throughput workflow from cDNA synthesis through library construction. This workstation has the capabilities to prepare 96 samples in one batch and includes elements for on deck incubations and purification workflows.

The biotechnology research company, Sampled, with sites in the US and UK, successfully processed different control inputs on the Sciclone G3 NGSx workstation. The resulting libraries aligned with QC and sequencing metrics expected of the PacBio® HiFiViral™ SARS-CoV-2 kit.

Materials

- Twist® Control 29 Delta
- Twist® Control 51 Omicron
- Illumina® COVIDSeq™ Positive Control
- PacBio® HiFiViral™ High-Throughput Multiplexing for Full-Viral Genome Sequencing of SARS-COV-2

Sciclone G3 NGSx Workstation

For research use only. Not for use in diagnostic procedures.

Experimental setup

The starting sample plate consist of 48 Illumina® human controls, 24 "Twist 29 Delta" controls and 24 "Twist 51 Omicron" controls. Each sample is diluted with RNase-free water to 200,000 copies in a 6uL volume.

Application method steps

The full workflow consists of two applications on the Sciclone G3 NGSx workstation with user touch points for off-deck incubation and reagent plating (figure 1). The HiFiViral™ App 1 has four steps, which take the samples all the way through cDNA amplification. The PacBio® HiFiViral™ App 2 has six steps on the Sciclone G3 NGSx workstation, which will take the pooled amplified cDNA through library construction.

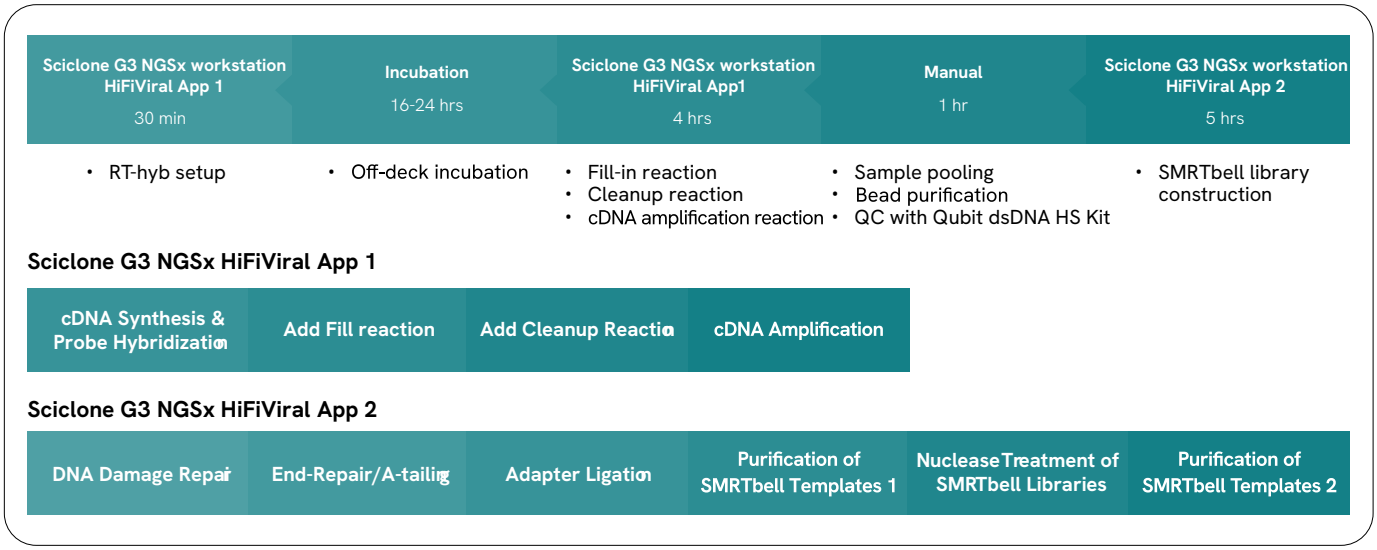
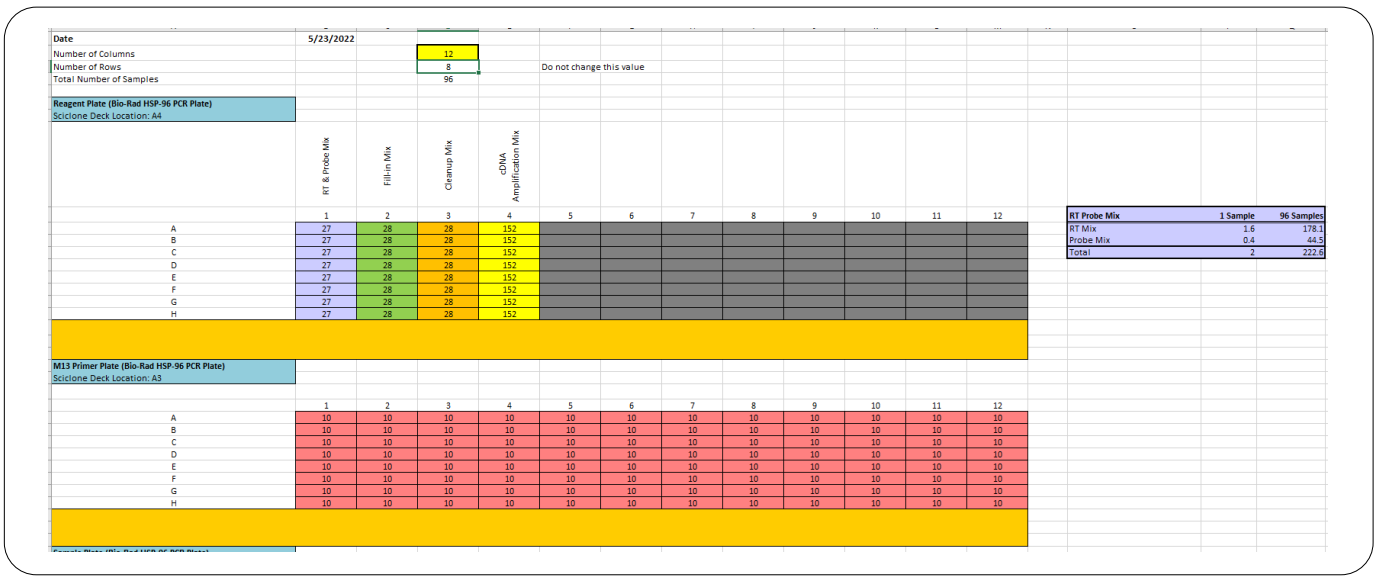


Figure 1. Full workflow of the PacBio® HiFiViral™ kit, including the workflow on the Sciclone G3 NGSx workstation.

To start each application, the user fills a reagent plate according to the volumes in the workbook, an excel file that calculates reagent volumes based on number of sample columns being processed (figure 2). The user will then proceed to start the application triggering prompts for the user to choose where to start the application, (figure 3), enters number of columns to process for a single run (figure 4) and setup images guiding the user through deck setup (figure 5).



Date	5/23/2022	
Number of Columns	12	
Number of Rows	8	Do not change this value
Total Number of Samples	96	

Reagent Plate (Bio-Rad HSP-96 PCR Plate)
Sciclone Deck Location: A4

	RT & Probe Mix	Fill-in Mix	Cleanup Mix	cDNA Amplification Mix
A	27	28	28	152
B	27	28	28	152
C	27	28	28	152
D	27	28	28	152
E	27	28	28	152
F	27	28	28	152
G	27	28	28	152
H	27	28	28	152

RT Probe Mix	1 Sample	96 Samples
RT Mix	1.6	178.1
Probe Mix	0.4	44.5
Total	2	222.6

M13 Primer Plate (Bio-Rad HSP-96 PCR Plate)
Sciclone Deck Location: A3

	1	2	3	4	5	6	7	8	9	10	11	12
A	10	10	10	10	10	10	10	10	10	10	10	10
B	10	10	10	10	10	10	10	10	10	10	10	10
C	10	10	10	10	10	10	10	10	10	10	10	10
D	10	10	10	10	10	10	10	10	10	10	10	10
E	10	10	10	10	10	10	10	10	10	10	10	10
F	10	10	10	10	10	10	10	10	10	10	10	10
G	10	10	10	10	10	10	10	10	10	10	10	10
H	10	10	10	10	10	10	10	10	10	10	10	10

Figure 2. The workbook for setting up the PacBio® HiFiViral™ Application.

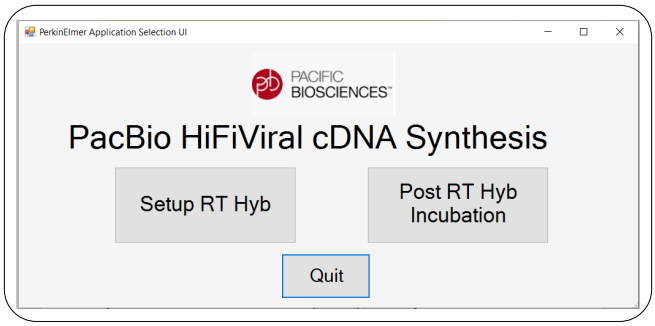


Figure 3: Application setup prompt for user.

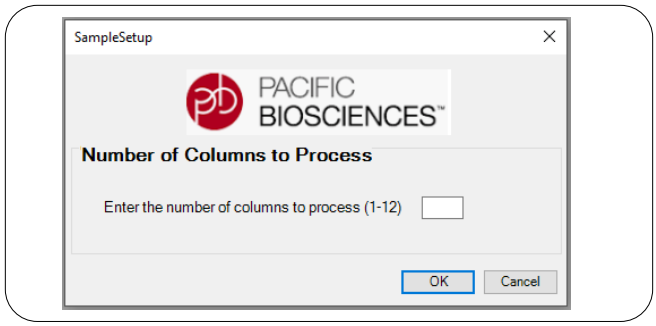


Figure 4: Sample setup prompt for user.

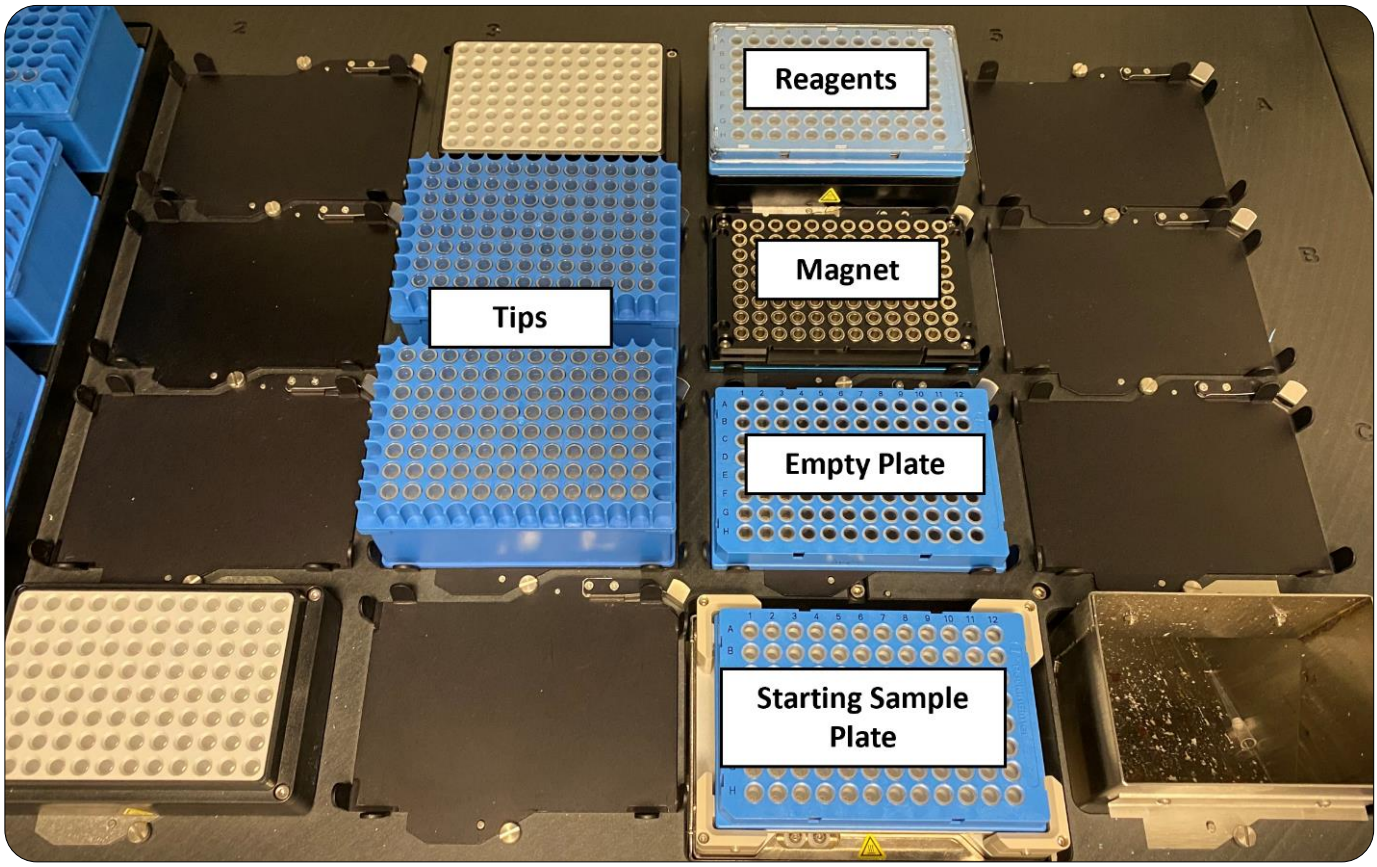


Figure 5: Full deck layout of Sciclone G3 NGSx HiFiViral™ App 1.

The Sciclone G3 NGSx workstation will start the liquid handling of the first application by broadcasting the RT reagent and probe mix from the reagent plate to the sample plate, mix, then prompt the user to remove the sample plate, seal, centrifuge and proceed to off deck hybridization for a minimum of 16 hours. Once hybridization is complete, the "Post RT Hyb Incubation" workflow is selected and plate is returned to deck when prompted. The instrument will continue to execute the method, adding in the fill-in mix, cleanup mix and PCR mastermix + barcodes. After each reagent addition, the user is prompted to seal, centrifuge, incubate off deck then resume the application when the plate is returned on deck. After the final addition of the PCR mastermix and barcode, the user will seal the plate and place it in a thermal cycler for cDNA amplification.

Once the plate is done thermal cycling, the user can quantify each sample with Thermo Fisher® Scientific Qubit® dsDNA HS kit and perform sample QC by running the sample through a nucleic acid analyzer. The samples are then manually pooled together by equal volume and undergo a manual bead purification with kit provided beads.

Post purification, the user will setup a plate with 500-1000 ng post-cleanup amplicon pool per well and place it on deck and setup the deck for the second Sciclone G3 NGSx application as pictured in figure 6.

The Sciclone G3 NGSx workstation will begin the liquid handling, broadcasting the DNA damage repair mix, end-repair/A-tailing mix, and adapter ligation mix with an incubation step after each reagent is added. Samples then go through a second and third purification using kit provided beads with a nuclease treatment in between.

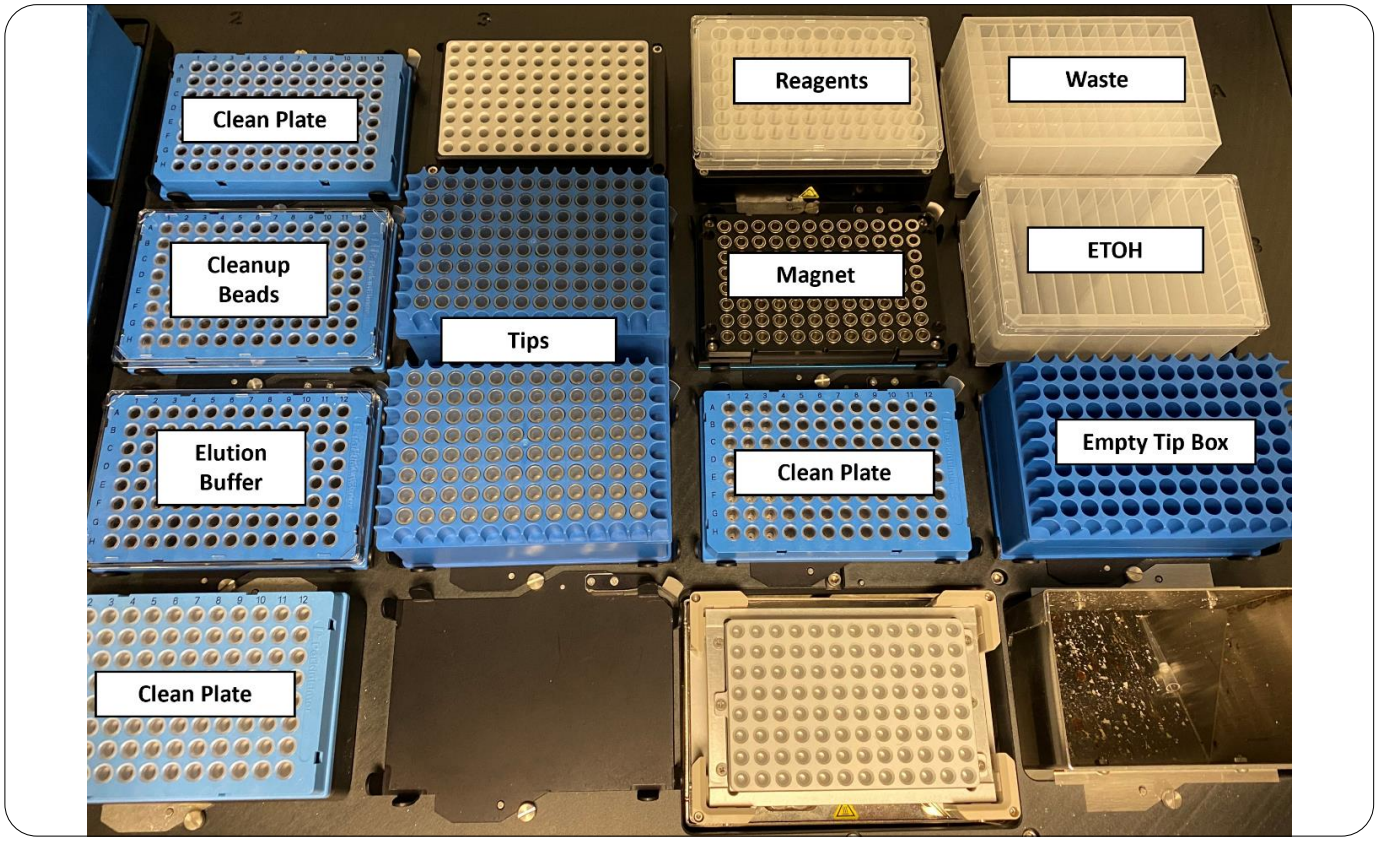


Figure 6: Deck layout of Sciclone G3 NGSx HiFiViral™ App 2.

Results

The full workflow produced PacBio® SMRTbell® libraries with yields from 8.76 ng/µL to 79.1 ng/µL (figure 7), averaging 55.6 ng/µL. All samples were within the target peak of > 700 bp. The final pooled library run on the on the LabChip® GX Touch™ nucleic acid analyzer was >1,000 bp as shown figure 8. Primary analysis results for two PacBio® HiFiViral™ libraries on the PacBio® Sequel lle are show in figure 9. With %P1 32%, the two libraries generated yield of 1.55 Gb with a mean subread length of 18.9 kb.

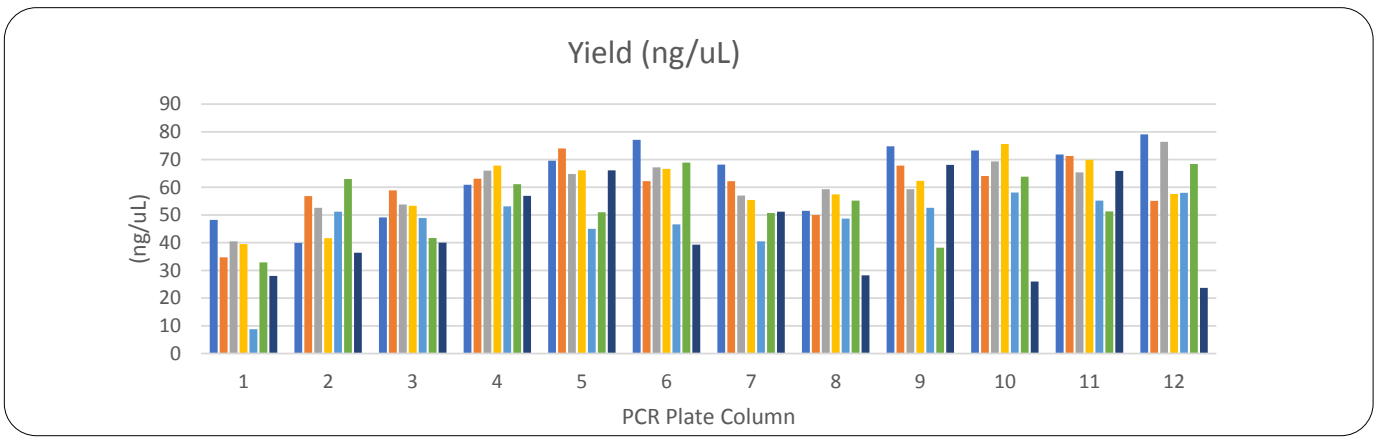


Figure 7: PacBio® SMRTbell® library yields (ng/uL)

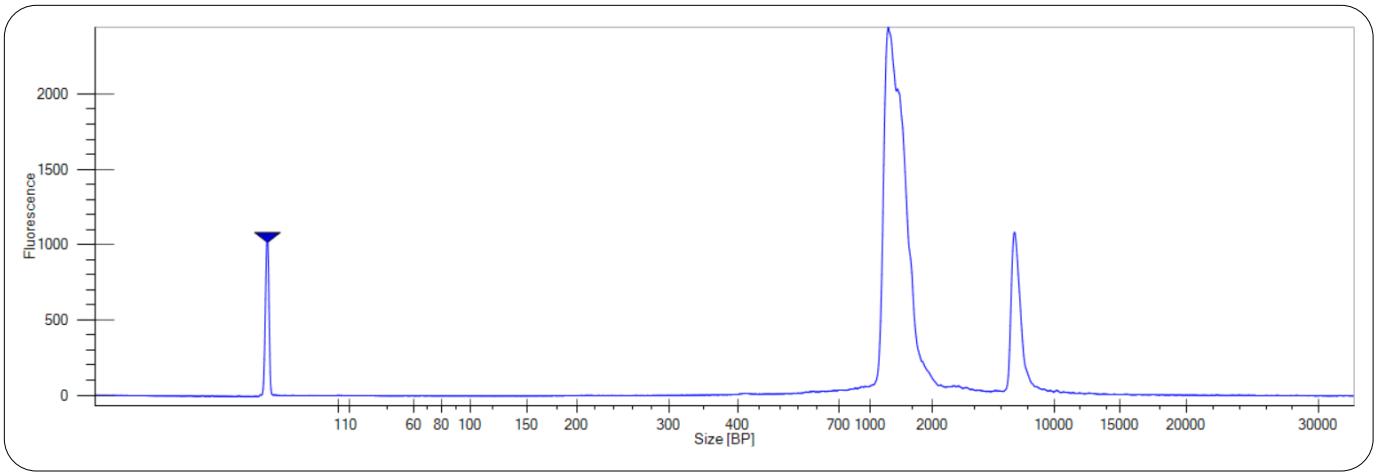


Figure 8: LabChip GX Touch electropherogram of pooled final library construction

	Sample Information	Run Settings				Productivity (%)			Reads				Control		Template
									HiFi Reads						
Well	Name	Movie Time (h...	Status	Total Bas...	Unique M...	P0	P1	P2	≥Q20 Reads	Yield	Mean Length	Median QV	Poly RL Me...	Local Base Rate	Adapter Dimer
A01	HiFiViral_Auto_84_24 (CCS)	8	Complete	78.72	3.82	66.1	32.0	1.9	1877725	1.55 Gb	824	Q60	18897	2.26	0

Figure 9: The results for control samples on a Sequel IIe prepared by Sciclone G3 NGSx workstation.

Conclusion

The PacBio® HiFiViral™ SARS-CoV-2 kit for detection of SARS-CoV-2 variants provides a robust, simple-to-use, scalable, cost-effective solution for sequencing the entire SARS-CoV-2 genome. Automating the PacBio® HiFiViral™ kit on the Sciclone G3 NGSx workstation not only reduced hands-on time and sample variability but also reduced the overall project cost. The Sciclone G3 System is intuitive and simple to use with its provided interface guided workflow set-up and step tracking.

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