APPLICATION NOTE

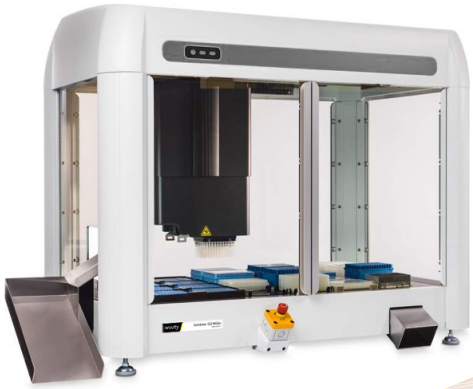
Strealiming PacBio® HiFi Library Prep using SMRTbell® Express Template Prep Kit 2.0.

Introduction

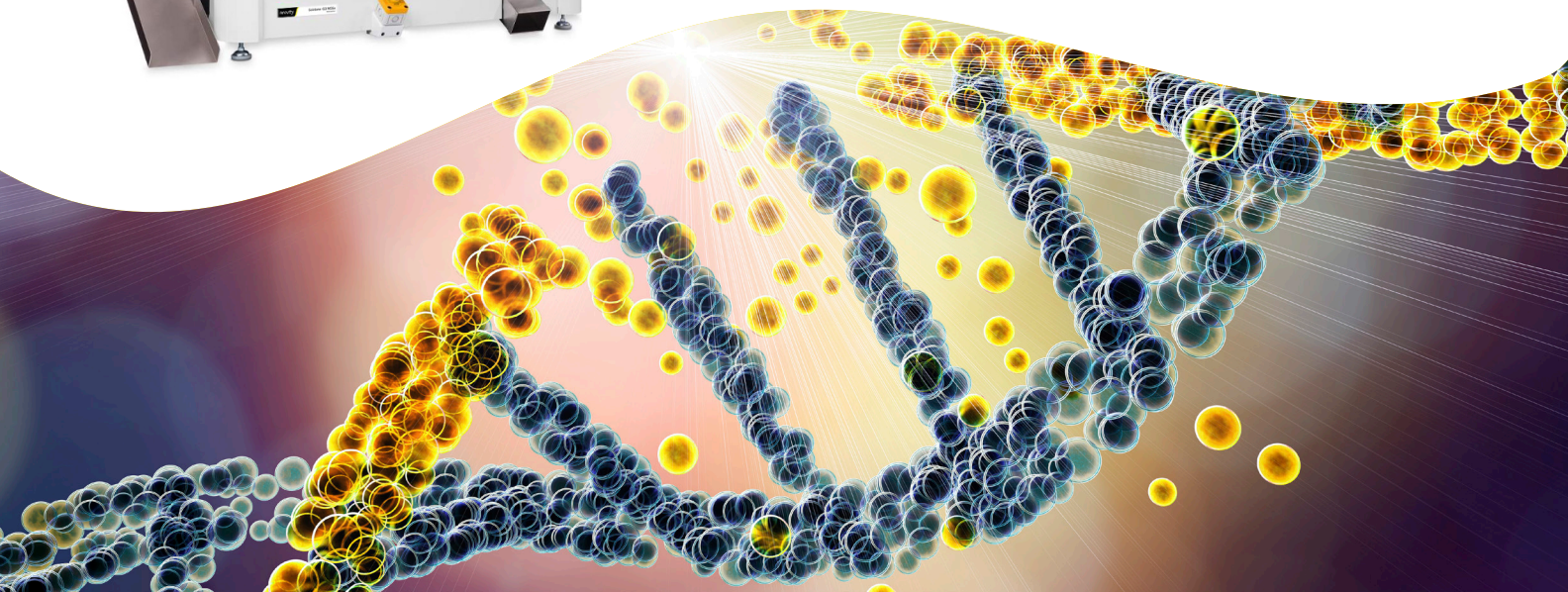
PacBio's SMRTbell® Express Template Prep Kit 2.0 provides a streamlined, single-tube library preparation workflow (PacBio Procedure 101-853-100) for generating SMRTbell® HiFi libraries, allowing easy automation on the Sciclone G3 NGSx workstation. This kit provides the ability to read tens of kilobases in length, as well as full-length transcripts, so the complete genome can be readily available.

The Sciclone G3 NGSx workstation is ideal for rapid and reliable NGS library construction. With the available pre-developed, standardized protocols, users can quickly automate their NGS workflows. Automating library construction workflows reduces hands-on time and variability, thereby improving overall project time and costs. This solution of the Sciclone G3 NGSx workstation and SMRTbell Express Template Prep Kit 2.0 enables users to load up to 96 sheared DNA samples and perform automated enzymatic reactions and purifications to generate SMRTbell libraries that can be size-selected on any commercially available size-selection systems.

Sciclone G3 NGSx Workstation



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



Experimental setup

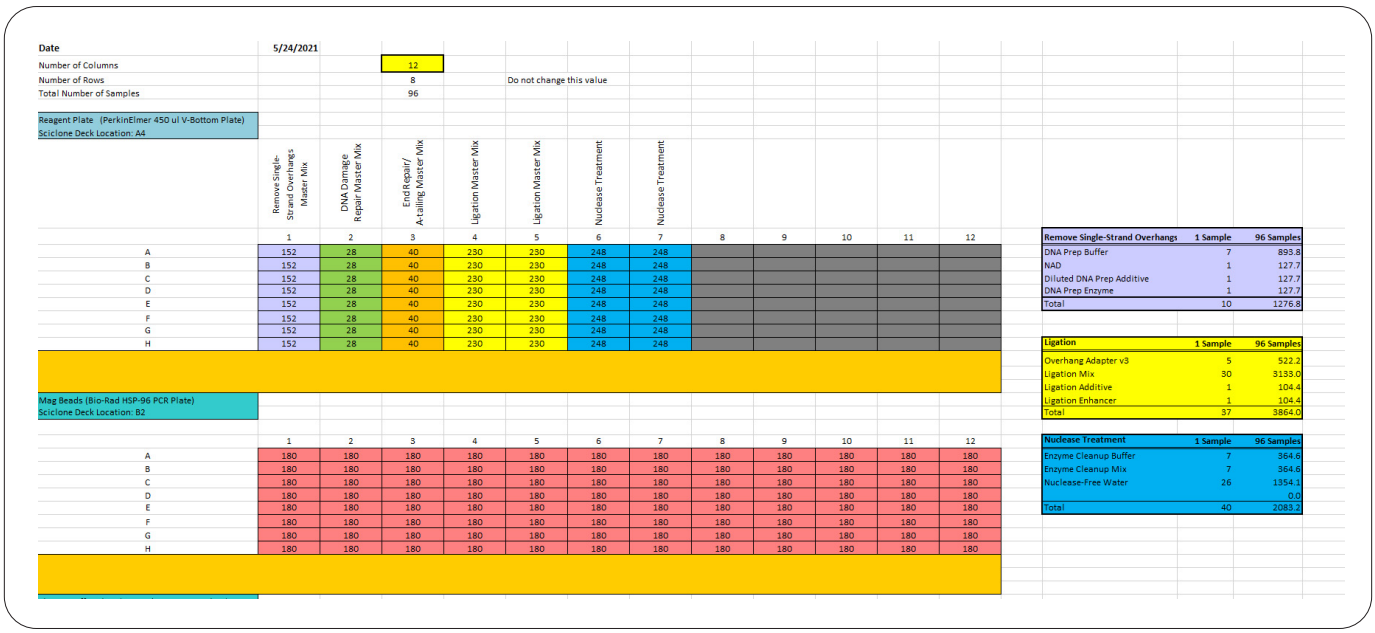
A set of 48 high-molecular weight genomic DNA samples, 28 E. coli and 20 human control samples, were prepared on the Sciclone G3 NGSx workstation using the workflow described in the separate document: Procedure & Checklist – Preparing HiFi SMRTbell® Libraries using the SMRTbell Express Template Prep Kit 2.0. The human samples were provided by Children's Mercy Hospital Kansas City. Genomic DNA samples were sheared using Diagenode® Megaruptor® 3 with the 2-cycle shearing protocol described in the PacBio HiFi procedure. Six micrograms of genomic DNA were sheared using speed 31 followed by a second cycle using speed 32. The recovered sheared DNA (between 50 -59 µL, 4.6 µg DNA) from the Megaruptor® 3 were used as input into the Sciclone G3 NGSx workstation.

Method steps

The HiFi Library prep application on the Sciclone G3 NGSx workstation consists of seven steps:

1. Remove Single Stranded Overhangs
2. DNA Damage Repair
3. End-Repair/A-Tailing
4. Adapter Ligation
5. Buffer Exchange
6. Nuclease Treatment
7. 1X Bead Cleanup

The run started with the user setting up the Sciclone G3 NGSx deck with consumables and reagents as shown in the provided workbook (figure 1) and setup images (figure 2). Master mix volumes were calculated by the Excel workbook based on the number of sample columns being processed. For 48 samples, a total of six columns were run. At the start of each run, a prompt is shown for the user to enter the number of columns to process (figure 3). Each of the master mixes are broadcast directly from the reagent plate on a chilled CPAC location to the sample plate. All incubations are completed on the on-deck CPAC locations. Prior to each incubation step, the user is prompted to seal and spin down the sample plate and then place the plate back on deck. For incubations with temperature changes, the plate is moved between the CPAC locations by the integrated gripper. After incubation steps, the user is prompted to spin the plate in a centrifuge and then place back on the deck without a seal. The total processing time for the library prep of 48 samples including incubations is approximately 6 hours.



Date	5/24/2021											
Number of Columns			12									
Number of Rows			8		Do not change this value							
Total Number of Samples			96									
Reagent Plate (PerkinElmer 450 ul V-Bottom Plate) Sciclone Deck Location: A4												
	Remove Single-Strand Overhangs Master Mix	DNA Damage Repair Master Mix	End Repair/ A-tailing Master Mix	Ligation Master Mix	Ligation Master Mix	Nuclease Treatment	Nuclease Treatment					
	1	2	3	4	5	6	7	8	9	10	11	12
A	152	28	40	230	230	248	248					
B	152	28	40	230	230	248	248					
C	152	28	40	230	230	248	248					
D	152	28	40	230	230	248	248					
E	152	28	40	230	230	248	248					
F	152	28	40	230	230	248	248					
G	152	28	40	230	230	248	248					
H	152	28	40	230	230	248	248					
Mag Beads (Bio-Rad HSP-96 PCR Plate) Sciclone Deck Location: B2												
	1	2	3	4	5	6	7	8	9	10	11	12
A	180	180	180	180	180	180	180	180	180	180	180	180
B	180	180	180	180	180	180	180	180	180	180	180	180
C	180	180	180	180	180	180	180	180	180	180	180	180
D	180	180	180	180	180	180	180	180	180	180	180	180
E	180	180	180	180	180	180	180	180	180	180	180	180
F	180	180	180	180	180	180	180	180	180	180	180	180
G	180	180	180	180	180	180	180	180	180	180	180	180
H	180	180	180	180	180	180	180	180	180	180	180	180

Remove Single-Strand Overhangs	1 Sample	96 Samples
DNA Prep Buffer	7	893.8
NAD	1	127.7
Diluted DNA Prep Additive	1	127.7
DNA Prep Enzyme	1	127.7
Total	10	1276.8

Ligation	1 Sample	96 Samples
Overhang Adapter v3	5	522.2
Ligation Mix	30	3133.0
Ligation Additive	1	104.4
Ligation Enhancer	1	104.4
Total	37	3864.0

Nuclease Treatment	1 Sample	96 Samples
Enzyme Cleanup Buffer	7	364.0
Enzyme Cleanup Mix	7	364.0
Nuclease-Free Water	26	1354.1
		0.0
Total	40	2083.2

Figure 1: The workbook for setting up the PacBio® HiFi Library Prep application.

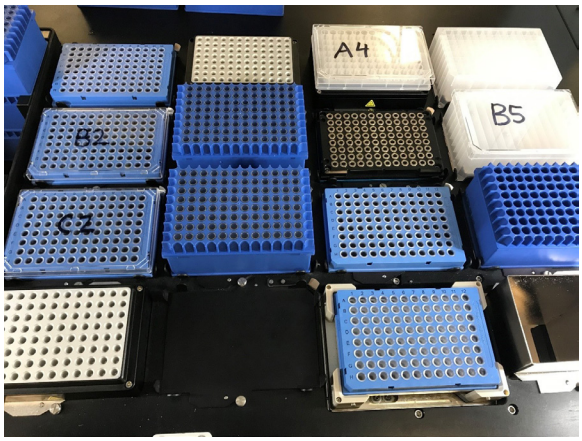


Figure 2: The deck layout to start the HiFi Library Prep application on the Sciclone G3 NGSx workstation.

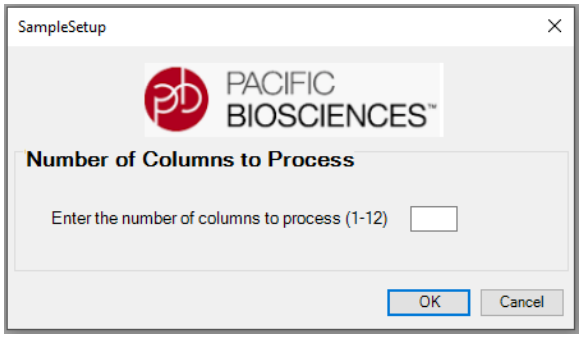


Figure 3: Sample setup prompt for user.

Results

The Sciclone G3 NGSx workstation produced SMRTbell® libraries with yields averaging 1.5 µg. These results fall within what is expected post-nuclease treatment, between 1 – 1.8 µg (red bars in figure 4).

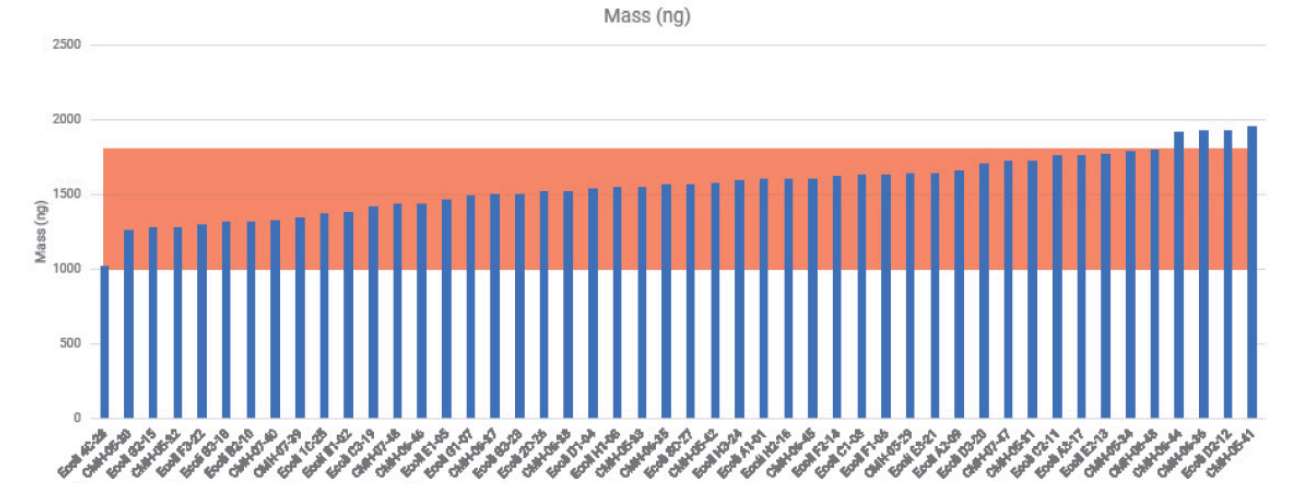


Figure 4: SMRTbell library yields generated by the Sciclone® G3 NGSx. Libraries may be size-selected on any commercially available size-selection systems. The shaded area in the chart represents the typical library yield from manual preparation.

The HiFi libraries were annealed with Sequencing Primer v2, bound with Sequel II Binding Kit 2.0 and sequenced on the PacBio® Sequel® IIe System at a concentration of 50 pM on-plate loading concentration. Movies were collected for 30 hours with 2-hour pre-extension time.

Primary Analysis results for two HiFi libraries are shown in Figure 5. With %P1 63.7% and 65.8%, the two libraries generated HiFi yield of 28.84 Gb and 25.64 Gb, with mean subread length of 18.4 kb and 17 kb. The data generated with automated library preparation are comparable in performance to manual preparation observed in this technical note.

	Sample Informati...	R.			Productivity (%)			Reads								Control		Template	
								HiFi Reads				Polymerase Read...		Longest Subread					
Well	Name	M...		Total ...	Unique...	P0	P1	P2	≥Q20 Reads	Yield	Mea...	Medi...	Mean	N50	Mean	N50	Poly RL ...	Local ...	Adap...	Sho...
A01	CMH 5 AUTO (CCS)	30	C..	381.26	94.96	34.3	63.7	2.0	1561982	28.84 Gb	18464	Q28	74720	156985	19355	22615	45763	1.91	0.01	0
B01	CMH 6 AUTO (CCS)	30	C..	357.97	83.22	33.0	65.8	1.3	1500269	25.64 Gb	17088	Q29	67955	153565	16389	19878	48231	1.87	0.01	0

Figure 5: The results for two Children Mercy Hospital samples on a Sequel II prepared by Sciclone G3 NGSx in March 2021.

Conclusion

SMRTbell library construction using PacBio's SMRTbell Express Template Prep Kit 2.0 kit was automated on the Revvity Sciclone G3 NGSx workstation to produce SMRTbell Libraries, ready for size-selection on commercially available size-selection systems. This automated solution which includes pre-developed and verified protocols, simplifies the end to end workflow for generating HiFi libraries for human variant detection on the PacBio Sequel IIe Systems.

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