APPLICATION NOTE

Automated PacBio® SMRTbell® Prep Kit 3.0 on Sciclone G3 NGSx for whole genome sequencing.

PacBio

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

Introduction

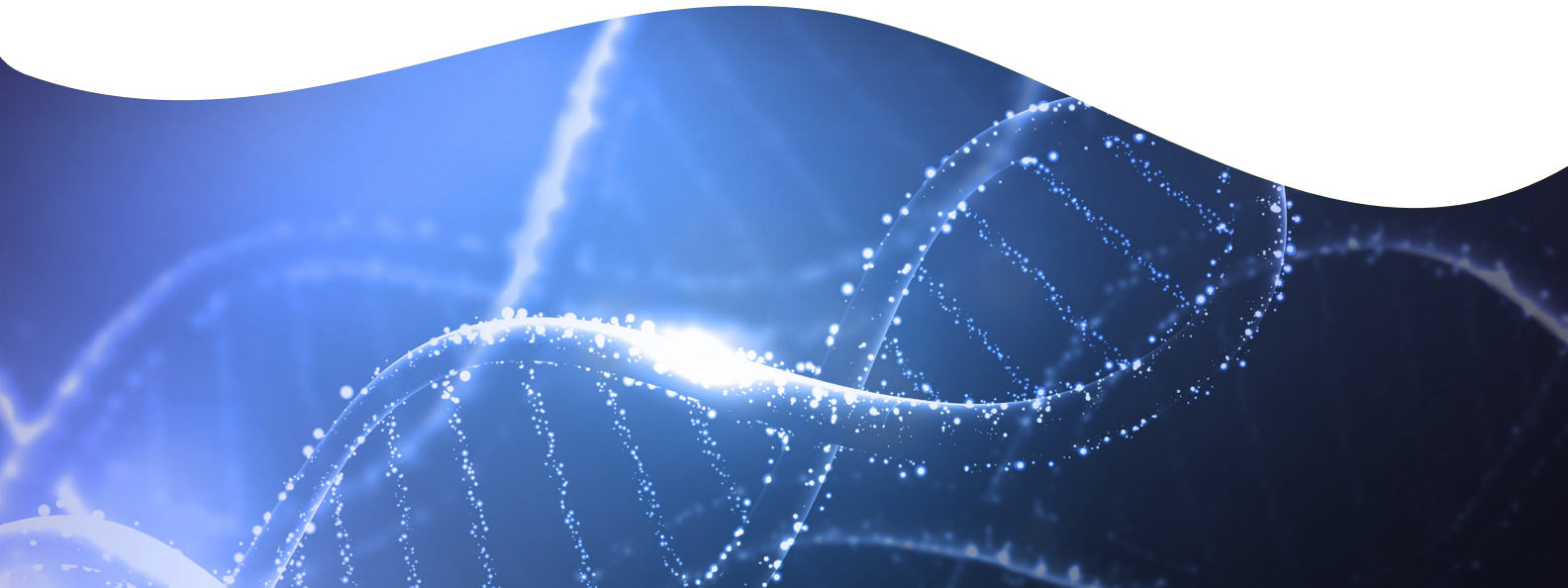
The PacBio® SMRTbell® Prep Kit 3.0 as automated on Revvity's Sciclone® G3 NGSx workstation offers a high throughput workflow for long-read library prep that reduces hands-on-time, human error, and variability, thereby reducing overall project costs. Users can load up to 96 sheared DNA samples on the Sciclone G3 system where enzymatic treatments are performed to generate SMRTbell libraries for whole genome sequencing. The Sciclone G3 system is intuitive, easy, and simple to use and provides an interface guided workflow set-up and step tracking.

Materials

HG001 cells
Nanobind® HT CBB kit (PN 102-301-900)
PacBio® SMRTbell® prep kit 3.0 (PN 102-182-700)

Experimental setup

DNA was extracted from HG001 cells using the protocol: Extracting HMW DNA using the Nanobind HT CBB kit for mammalian cultured cells on the KingFisher Apex system. The extracted DNA was then sheared at PacBio resulting in a mode size of 19,569 bp. Following shearing, the sample went through a concentration and cleanup step with 1X SMRTbell cleanup beads. After cleanup, samples were plated into 48 wells in a checkerboard pattern (figure 1) with each well containing 2 µg of total of sheared DNA. The other 48 wells were filled with DNase/RNase free water samples for non-template controls (NTC).



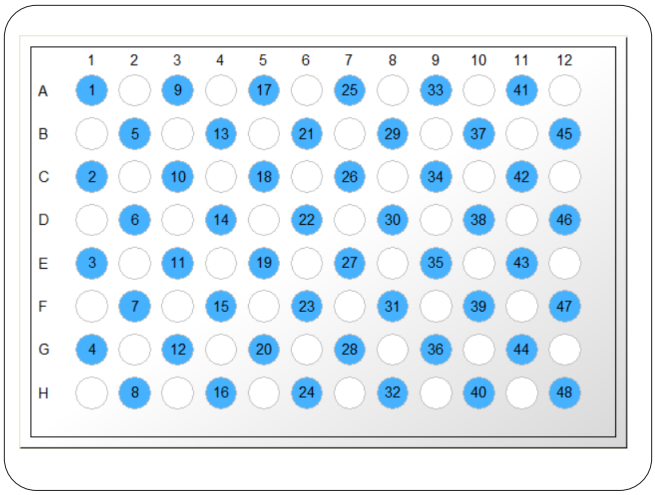


Figure 1. Checkerboard pattern showing the wells with samples.

Application method steps

Add End Repair and A-Tailing master mix to samples
(20 minutes)

End Repair and A-Tailing Incubation off-deck
(35 minutes)

Add Adapter Ligation master mix to samples and incubate on deck for 30 minutes.
(50 minutes)

Perform 1X cleanup with SMRTbell Cleanup Beads
(60 minutes)

Add Nuclease Treatment master mix to samples
(20 minutes)

Incubate Nuclease Treatment off-deck
(15 minutes)

Perform 1X Cleanup with SMRTbell cleanup beads or AMPure® PB Beads size selection
(60 minutes)

Figure 2. The PacBio SMRTbell Prep kit 3.0 application on the Sciclone G3 NGSx Workstation.

To start the application, the user fills the reagent plates according to the volumes and plate type from the workbook, an excel file that calculates reagent volumes based on number of sample columns being processed (figure 3). The user then proceeds to start the application triggering prompts for the user to start the application, (figure 4), enter the number of columns to process for a single run,choose options for on/off deck incubation (figure 5) and setup images guiding the user through deck setup (figure 6).

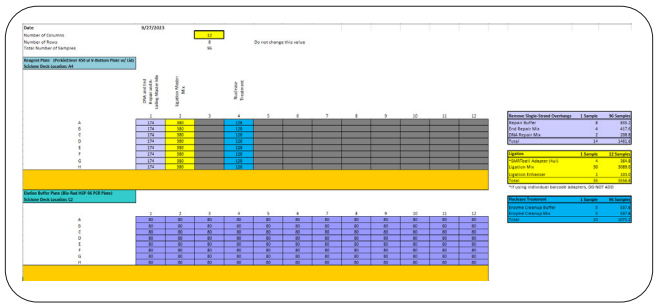


Figure 3. The workbook for setting up the PacBio SMRTbell prep kit 3.0 application.

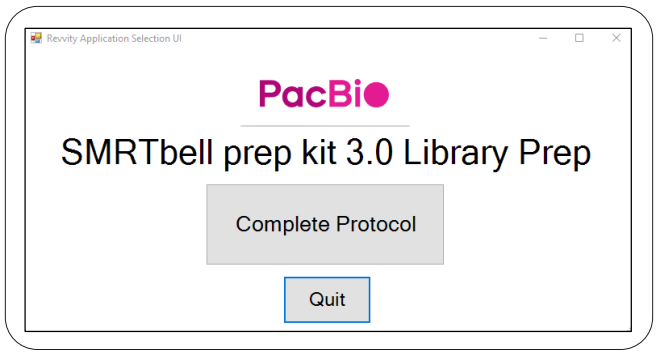


Figure 4. Application setup prompt for user.

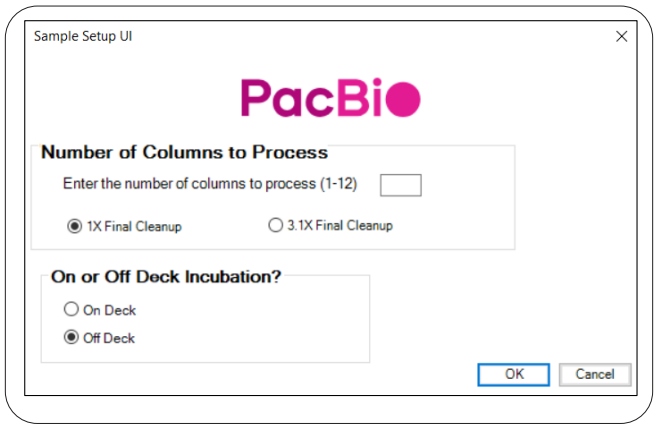


Figure 5. Sample setup prompt for user.

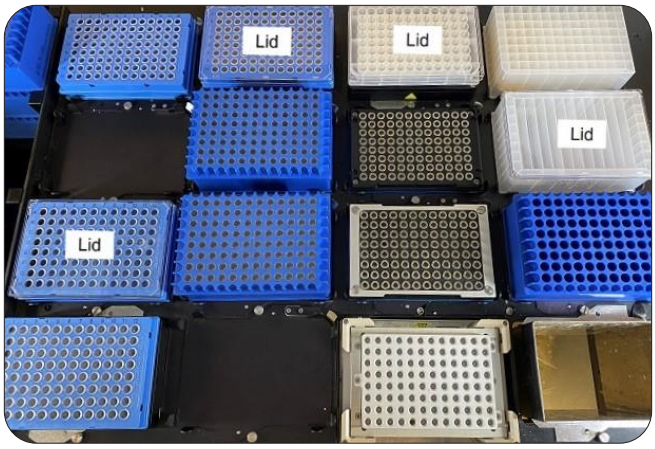


Figure 6. Full deck layout of the application with 1X final SMRTbell cleanup option.

The Sciclone G3 NGSx workstation will start the liquid handling of the first application by broadcasting the ER/A-tailing master mix from the reagent plate to the sample plate, mix, then prompt the user to remove the sample plate, seal and proceed to an on/off deck incubation at 37°C for 30 minutes, then 65°C for 5 minutes. Once ER/A-tailing incubation is complete, the plate is placed back on the instrument and the liquid handling will continue by broadcasting ligation master mix to sample, mix, then proceeding to on deck incubation on the thermoshake at 20°C for 30 minutes. Post ligation, the samples will go through a 1X bead cleanup using provided kit SMRTbell cleanup beads. Once cleanup is complete, the nuclease treatment is broadcast to the sample and the user is prompted again to seal the sample plate and proceed to on/off deck incubation at 37°C for 15 minutes. When the incubation is complete a second round of purification is performed with 1X SMRTbell cleanup beads or with the AMPure PB beads at 3.1X ratio.

Sequencing methods on PacBio Sequel® IIe and Revio™ systems

To demonstrate sequencing performance on the Revio system, as well as the ability to multiplex, an equal volume, 6 µL, was pooled from sample wells A7, C5, and E5. The pool was then size selected on the Pippin HT using a 10 kb cutoff. A total of 757 ng of library was recovered representing a 67% size selection yield. The pooled library was then prepared for sequencing using the Revio polymerase kit (PN 102-817-600) and loaded at 215 pM, 2-hour pre-extension and 24-hour movie time. The remaining samples (n = 44, well D6 was lost because of evaporation during shipping) were pooled, using an equal volume, followed by a 1X SMRTbell cleanup step. The 44-plex pool was prepared for sequence using the Sequel II binding kit 3.2 (PN 102-333-300) and loaded at 85 pM using 2-hour pre-extension and 30-hour movie time.

Results

The full workflow using the 1X SMRTbell cleanup bead option produced libraries with an average yield of 54.91 ng/µL (figure 7) with an average of 38.93% recovery which is within the expected range of the kit. Sequenced libraries demonstrated high base yields on Revio and Sequel IIe systems. The 3-plex pool on the Revio system yielded a total of 98 Gb of HiFi bases with mean read lengths >19 kb for each of the three demultiplexed samples (Table 1 and Figure 9). Whereas the 44-plex pool on Sequel IIe yielded a total 35 Gb, with good coverage balance across all 44 samples (Figure 10). Mean read lengths on the 44-plex pool were lower, at 16 kb, because the samples were not size selected.

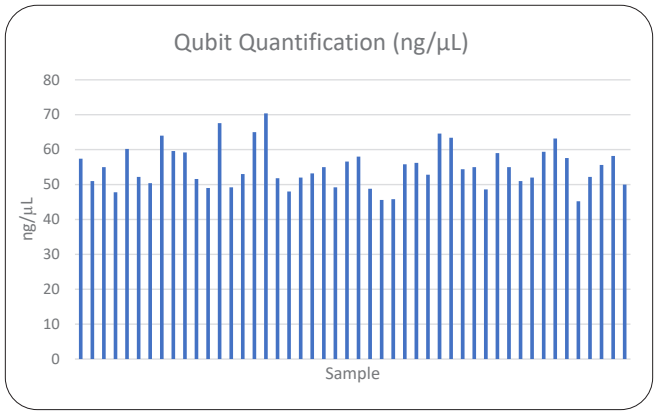
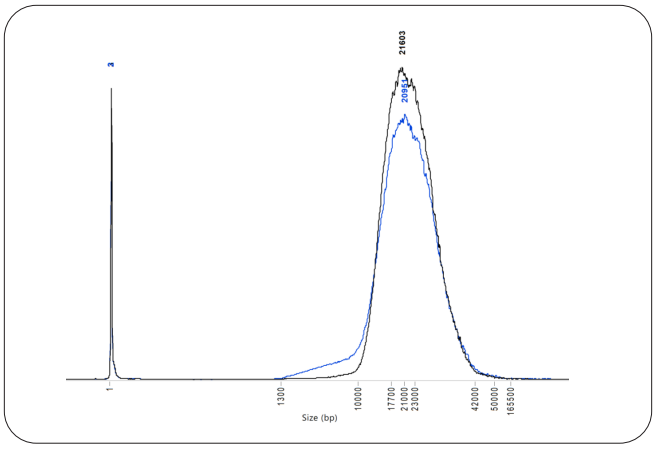


Figure 7. SMRTbell library yields (ng/µL)

Conclusion

By automating the PacBio SMRTbell kit 3.0 on the Sciclone G3 NGSx workstation, an additional 4.5 hours of hands-on-time can be eliminated from the workflow, thus simplifying the workflow process, and reducing experiment time. Please contact your local Revvity representative for more information.



Sample	Color	Average size
3 plex pre size selection	Blue	20,951 bp
3 plex post Pippin HT size selection	Black	21,603 bp

Figure 8. Electropherogram of final library construction

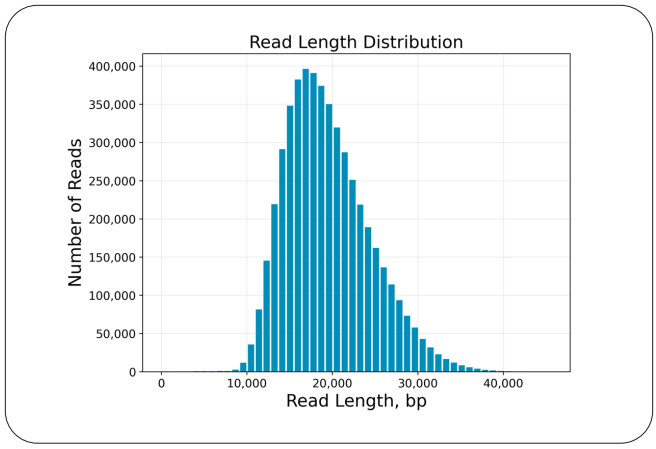


Figure 9. HiFi read length distribution of three-sample pool size selected on the Sage Science Pippin HT and sequenced on a single Revio SMRT® Cell. Libraries were prepared on the Sciclone G3 NGSx workstation. Average HiFi read length was 19,468 bp.

Table 1. Revio sequencing metrics for the three sequenced barcoded samples prepared on the Sciclone G3 NGSx system. The three samples were pooled in an equal volume, size selected on the Sage Science Pippin HT system, and sequenced on a single Revio SMRT Cell. The run achieved a total of 98 Gb of barcoded HiFi data, and > 10-fold coverage across the human genome for each sample.

Sample	Barcode	HiFi Reads	HiFi Read Length mean	HiFi Read Quality mean	HiFi Yield (Gb)	Fold coverage per sample
HG001 C5	bc2035	1,684,696	19,488 bp	Q27	32.83	10.5
HG001 E5	bc2037	1,665,826	19,517 bp	Q27	32.51	10.4
HG001 A7	bc2049	1,690,932	19,407 bp	Q27	32.82	10.5
NA	No barcode	43,647	19,166 bp	Q24	00.84	na

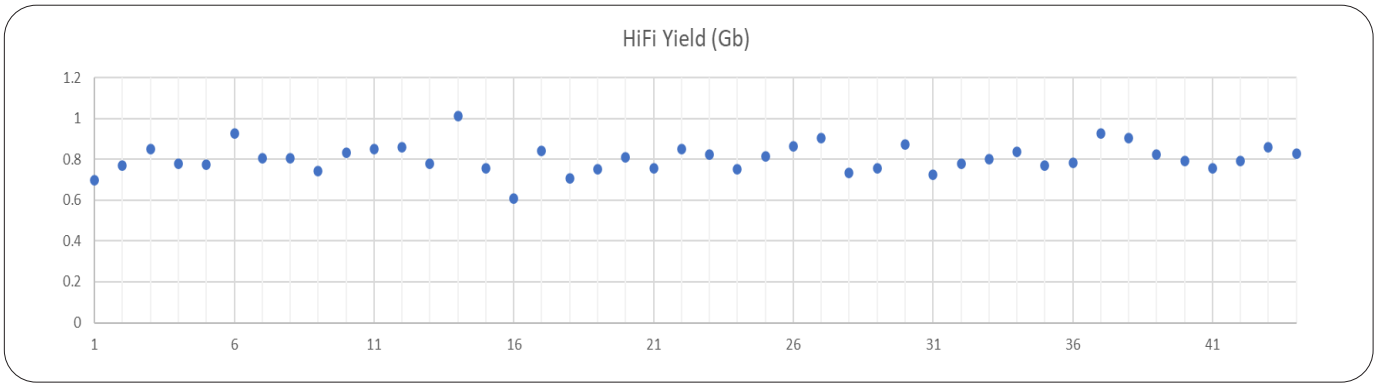


Figure 10. HiFi yield per barcoded library prepared on the Sciclone G3 NGSx system (n= 44) and sequenced on the Sequel IIe system. The 44-plex pool yielded 35.45 Gb of HiFi data and all barcodes were detected.

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