Introduction

PacBio’s SMRTbell® (Single Molecule, Real-Time Sequencing) Express Template Prep Kit 2.0 provides a streamlined, single-tube library preparation workflow 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 NGS 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.

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.

![Figure 1. The workbook for setting up the PacBio® HiFi Library Prep application.](image)

![Figure 2. The deck layout to start the HiFi Library Prep application on the Sciclone G3 NGSx workstation.](image)
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.

---

**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).

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.

---

**Figure 3:** Sample setup prompt for user.

**Figure 4:** SMRTbell library yields generated by the Sciclone® G3 NGSx. Libraries may be size-selected on any commercially available size-selection systems.

**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 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 PerkinElmer 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 Ile Systems.