Automating PacBio® SMRTbell® Whole Genome Sequencing Library Preps on Miro Canvas®

Data from a demonstrated protocol

Key points

- Whole genome sequencing (WGS) library preparation with PacBio's SMRTbell Express Template Prep Kit 2.0 and SMRTbell Prep Kit 3.0 is fully automated on Miro Canvas using 1-5 µg high quality, high molecular weight input DNA
- These protocols offer the flexibility to choose an automated, fast bead-based size selection, or a more stringent gel-based size selection
- For both protocols, Miro Canvas total library quantities, peak sizes, and primary sequencing metrics are indistinguishable from manually prepared libraries

Introduction

Long read sequencing plays an important role in generating contiguous, high-quality genomes, for haplotype phasing, structural variant detection, and de novo assemblies[1]. Additionally, long read libraries that are prepared without PCR amplification avoid a common source of base composition bias in sequencing data[2]. Many long read library prep workflows, such as with the SMRTbell Express Template Prep Kit 2.0, have traditionally used a gel-based size selection of the library to efficiently remove small molecules. However, this type of size selection generally requires large DNA inputs and is not automatable. The PacBio SMRTbell Prep Kit 3.0 combines these advantages of PCR-free long read sequencing with a streamlined protocol and fast bead-based size selection, for an easily automated long read library preparation.

Miro Canvas is a digital microfluidics (DMF) platform that allows low-throughput workflow automation for complex protocols such as NGS library preparation. The system is compatible with a wide range of reagents, and as such, kits from both Miroculus and other reagent suppliers can be used. This application note describes the results that can be expected when using the SMRTbell Express Template Kit 2.0 and SMRTbell Prep Kit 3.0 in protocols developed for Miro Canvas. The resulting research use only libraries can then be sequenced using the PacBio sequencing systems.

Experimental workflow

The SMRTbell Express Template Kit 2.0 and SMRTbell Prep Kit 3.0 protocols were designed with automated systems such as the Miro Canvas in mind and have been tested using high quality, high molecular weight 1-5 µg DNA inputs. Before beginning, DNA should be fragmented to 15-18 kb using a Megaruptor, and quantified before and/or after fragmentation using a Broad-Range Qubit quantification kit or similar. For SMRTbell Prep Kit 3.0, post-shearing cleanup, repair and A-tailing, adapter ligation, post-ligation cleanup, nuclease treatment, and bead-based size selection steps are all automated on Miro Canvas (Fig.1), and result in a ready-to-sequence library. For SMRTbell Express Template Prep Kit 2.0, single strand overhang removal, DNA damage repair, end repair and A-tailing, adapter ligation, post-ligation cleanup, nuclease treatment, and post-nuclease cleanup steps are all automated on Miro Canvas (Fig.1), however, an off-Canvas gel-based selection and subsequent cleanup step are required prior to sequencing.

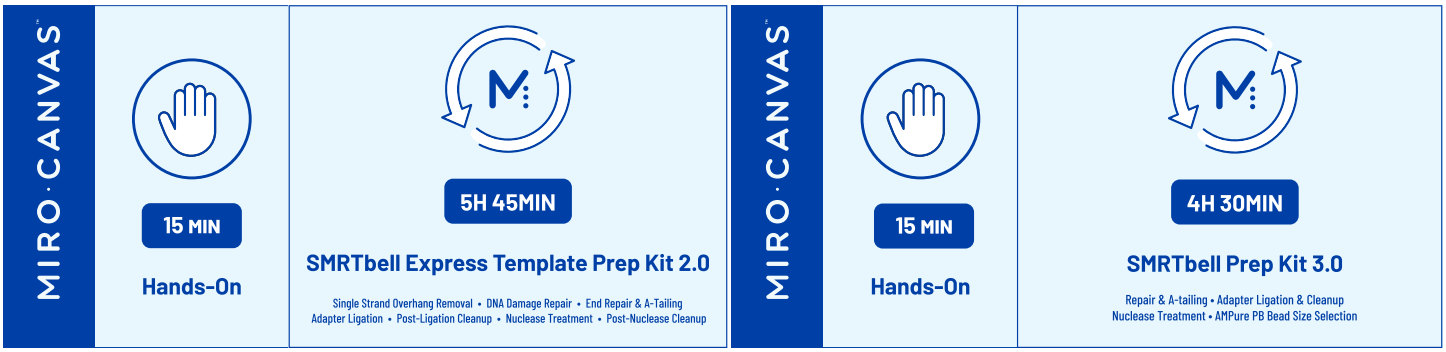


Figure 1. Experimental Workflows. For SMRTbell Prep Kit 3.0, the Miro Canvas automates all of the steps following reaction setup. For SMRTbell Express Template Prep Kit 2.0, a gel-based size selection step and subsequent cleanup are required following Miro Canvas automation.

Results

SMRTbell Library Preparations Automated on the Miro Canvas Produce Comparable Total Library Quantity

SMRTbell libraries were constructed with 1-5 µg of high quality, high molecular weight NA24385 (HG002)* DNA. Final library quantities for both manually-prepared and Miro Canvas-prepared libraries were assessed using Qubit Broad-Range or Qubit High Sensitivity kits. For each of the kits evaluated, the Miro Canvas produced comparable libraries to manual preparation (Table 1).

*NA24385 DNA was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research

		Total Library (ng)	
	Input DNA	Manual	Miro Canvas
SMRTbell Express Template Prep Kit 2.0	5µg	692 +/- 218	607 +/- 61
SMRTbell Prep Kit 3.0	1µg	182 +/- 16	159 +/- 16

Table 1. SMRTbell Express TemplatePrep Kit 2.0 and SMRTbell Prep Kit 3.0 libraries performed comparably on the Miro Canvas, as compared to manually-prepared libraries. Total library quantity and peak size are shown as: average +/- standard deviation (n=6).

Efficient Removal of Small Library Molecules Using Diluted Bead-Based Size Selection in the SMRTbell Prep Kit 3.0

Diluted bead-based size selection offers many advantages over traditional gel-based size selection of long read libraries: automatability, workflow time, and input requirements. The SMRTbell Prep Kit 3.0 protocol on the Miro Canvas constructed libraries from just 1 µg of input and efficiently removed small library molecules with an automated bead-based size selection (Fig 2).

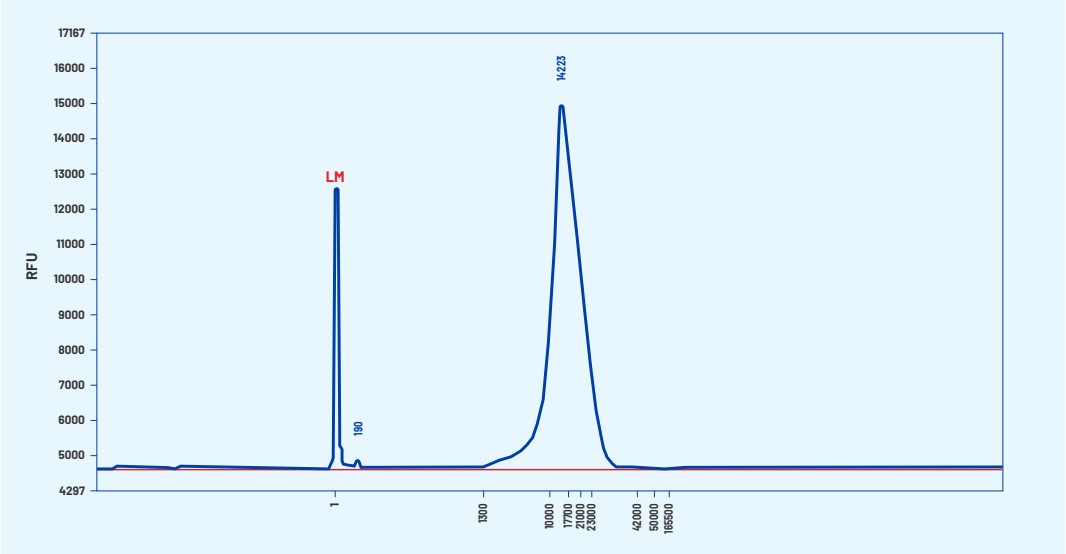
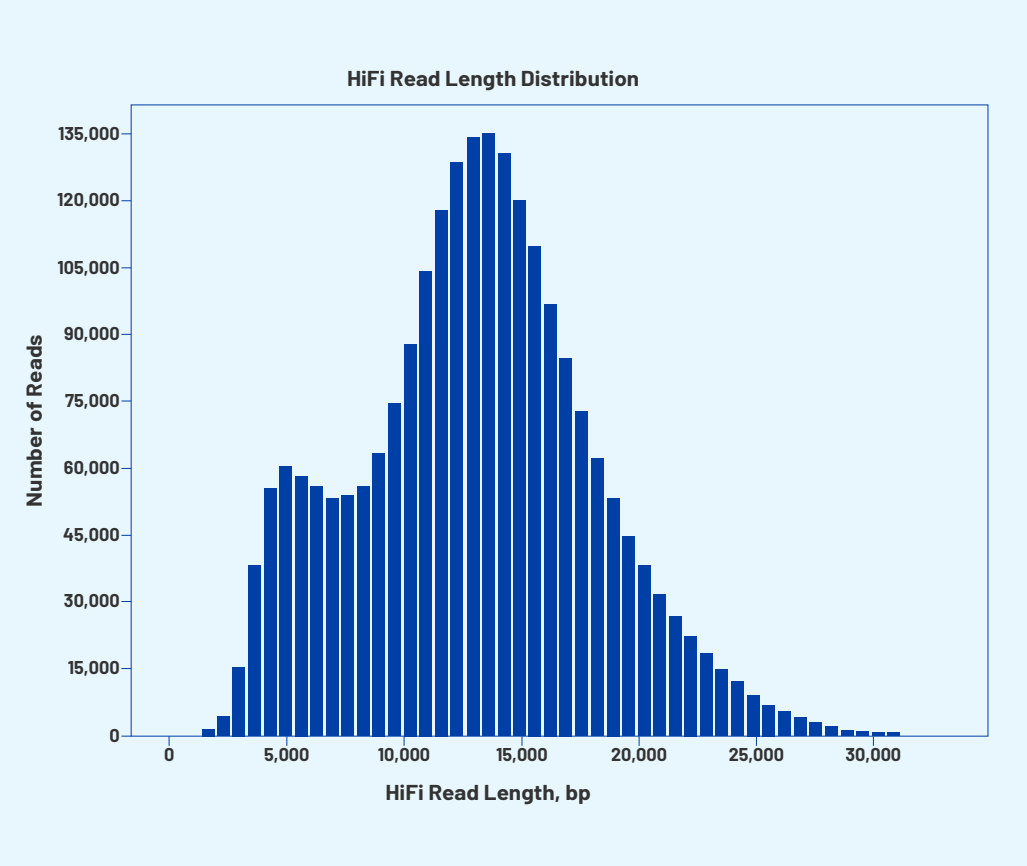


Figure 2. SMRTbell Prep Kit 3.0 library size distribution. Following automation on the Miro Canvas with a bead-based size selection, libraries were examined on a Femto Pulse (Agilent) to demonstrate efficient removal of small library molecules.

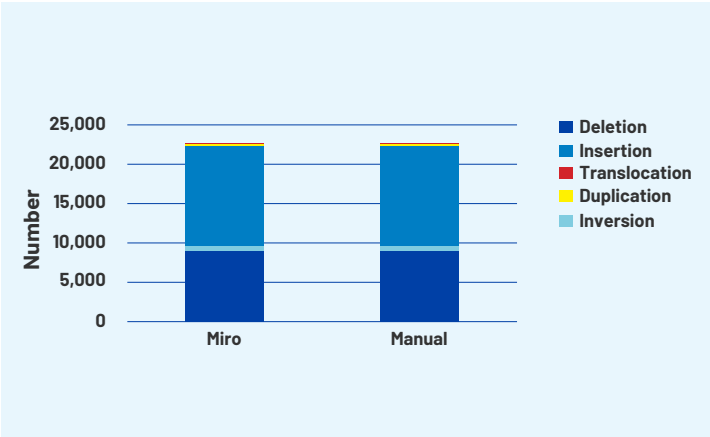
Sequencing Metrics from Miro Canvas and Manually-Prepared Libraries Indicate Comparable HiFi Yield, Read Length, Read Quality, and Structural Variant Detection

SMRTbell libraries were sequenced on a Sequel® II System using Binding Kit 2.2, Sequencing Kit 2.0, and 30 hour movies. This demonstrated equivalency across manual and automated library preps. Of particular note, the new SMRTbell Prep Kit 3.0 not only requires less input material and eliminates the need for cumbersome gel-based size selection methods, it also results in libraries with excellent sequencing performance (Fig 3) and structural variant detection (Fig 4).



Value	Analysis Metrics
2,268,270	HiFi Reads
29,567,143,518	HiFi Yield (bp)
13,035	HiFi Read Length (mean, bp)
Q37	HiFi Read Quality (Median)

Figure 3. SMRTbell Prep Kit 3.0 sequencing metrics. Miro Canvas yield, read length and read quality metrics are all equivalent to manually prepared libraries.



	Miro Canvas	Manual
Deletion	9,070	9,174
Duplication	446	442
Insertion	12,586	12,437
Inversion	88	94
Translocation	122	162
Total	22,312	22,309

Figure 4. SMRTbell Prep Kit 3.0 structural variant detection. Miro Canvas detection of deletions, duplications, insertion, inversions and translocations in a NA24385 (HG002) DNA sample are all comparable to manually prepared libraries.

Summary

Miro Canvas is an advanced DMF platform that can be used to automate library preparation with the PacBio SMRTbell Express Template Prep Kit 2.0 and SMRTbell Prep Kit 3.0. When using the SMRTbell Prep Kit 3.0 for Miro Canvas, the protocol is fully automated from post-shear cleanup to elution. When using the SMRTbell Express Template Prep Kit 2.0 for Miro Canvas, the protocol is automated from single strand overhang removal to post-nuclease cleanup elution, requiring an off-Canvas gel-based size selection step and subsequent cleanup. Both Miro Canvas and manual library preparation yield high quality libraries with comparable sequencing performance and structural variant detection.

References

1. Wenger AM, Peluso P, Rowell WJ, Chang P, Hall RJ, Concepcion GT, Ebler J, Fungtammasan A, Kolesnikov A, Olson ND, Töpfer A, Alonge M, Mahmoud M, Qian Y, Chin C, Phillippy AM, Schatz MC, Myers G, DePristo MA, Ruan J, Marschall T, Sedlazeck FJ, Zook JM, Li H, Koren S, Carroll A, Rank DR, Hunkapiller MW. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nature Biotechnology. 2019 Oct;37(10):1155-1162. doi: 10.1038/s41587-019-0217-9. Epub 2019 Aug 12. PMID: 31406327.
2. Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. Nature reviews. Genetics. 2020 Oct;21(10):597-614. doi: 10.1038/s41576-020-0236-x. Epub 2020 Jun 5. PMID: 32504078.

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Revision History

Version
1.0
Revisions
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