Application Note

Automating PacBio® SMRTbell® whole genome sequencing library prep on MIRO CANVAS®

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, have traditionally used a gel-based size selection to efficiently remove small molecules from the library.

However, this type of size selection generally requires large DNA inputs and is not automatable. The PacBio SMRTbell Prep Kit 3.0 combines the 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. This application note describes the results that can be expected when using the SMRTbell Prep Kit 3.0 in a protocol developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using the PacBio sequencing systems.

Key benefits:

- Whole genome sequencing (WGS) library preparation with PacBio's SMRTbell Prep Kit 3.0 is fully automated on the MIRO CANVAS using 1-3 µg of high quality, high molecular weight input DNA.
- This protocol offers the flexibility to choose automated, fast bead-based size selection, or a more stringent gel-based size selection.
- For the SMRTbell Prep Kit 3.0 protocol, MIRO CANVAS total library quantities, peak sizes and primary sequencing metrics are indistinguishable from manually prepared libraries.

Overview: How to automate PacBio SMRTbell WGS on MIRO CANVAS

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Experimental set-up

The SMRTbell Prep Kit 3.0 protocol was designed with automated systems – such as the MIRO CANVAS – in mind, and have been tested using high quality, high molecular weight 1-3 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 the SMRTbell Prep Kit 3.0, post-shearing clean-up, repair and A-tailing, adapter ligation, post-ligation clean-up, nuclease treatment and bead-based size selection are all automated on MIRO CANVAS (**Figure 1**), resulting in a ready-to-sequence library.

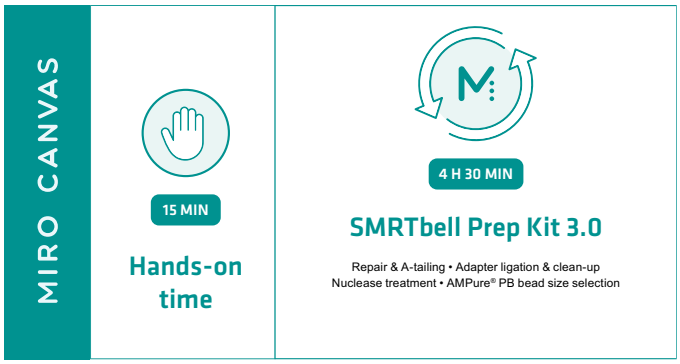


Figure 1: Experimental set-up. For the SMRTbell Prep Kit 3.0, the MIRO CANVAS automates all of the steps following reaction set-up.

Results

SMRTbell libraries were constructed with 1 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 broad range or high sensitivity Qubit 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.

Table 1: SMRTbell Prep Kit 3.0 libraries generated with the MIRO CANVAS were comparable to manually prepared libraries. Total library quantity shown as average +/- standard deviation (n=6).

		Total library (ng)	
	Input DNA	Manual	MIRO CANVAS
SMRTbell Prep Kit 3.0	1 µg	182 ±16	159 ±16

Efficient removal of small molecules from libraries using diluted bead-based size selection with the SMRTbell Prep Kit 3.0

Diluted bead-based size selection offers many advantages over traditional gel-based size selection of long read libraries, including automatability, reduced workflow times, and lower input requirements. Performing the SMRTbell Prep Kit 3.0 protocol on the MIRO CANVAS enabled construction of libraries from just 1 µg of input material, and efficiently removed small molecules from the library with automated bead-based size selection (**Figure 2**).

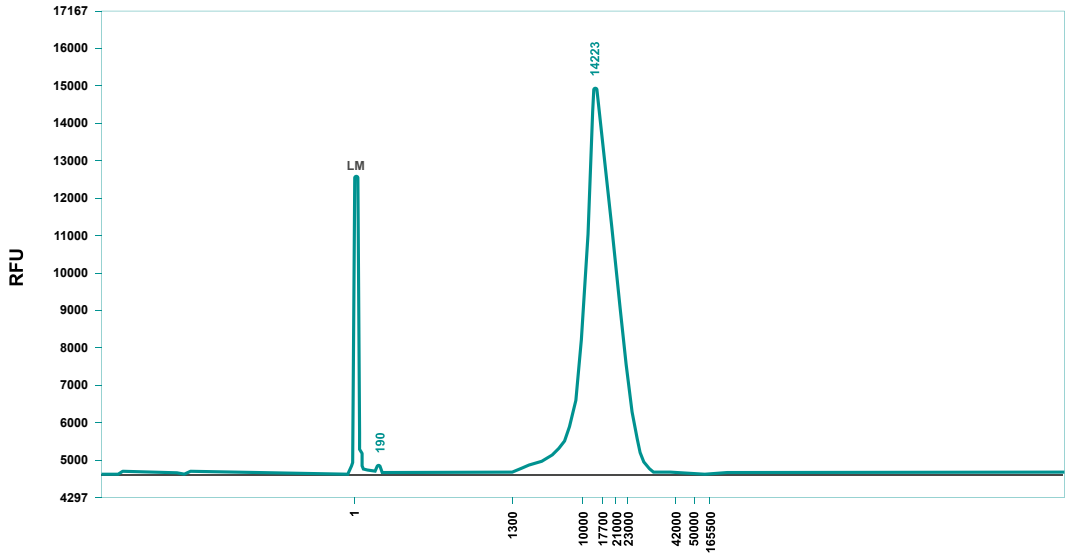
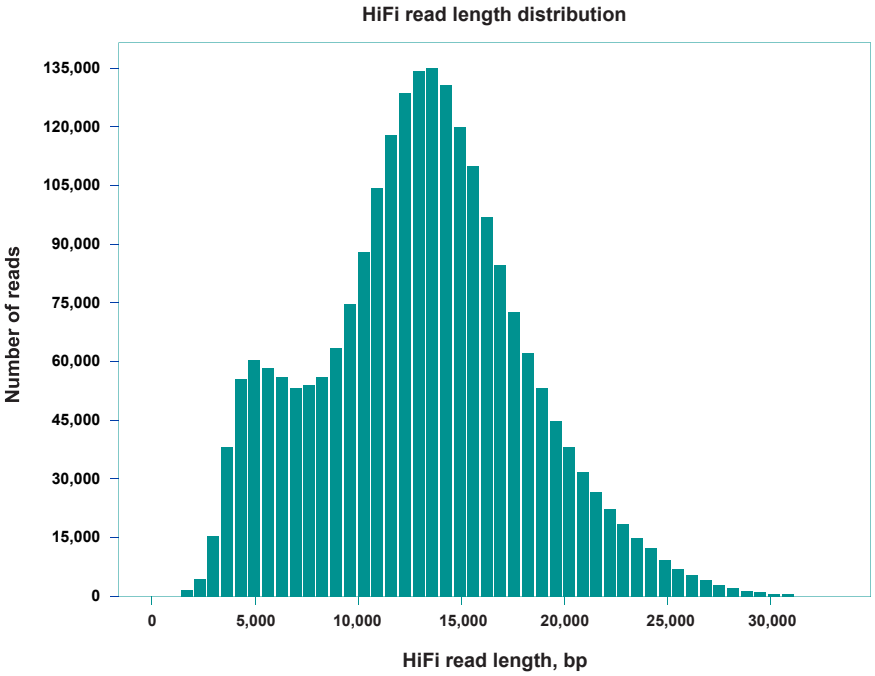


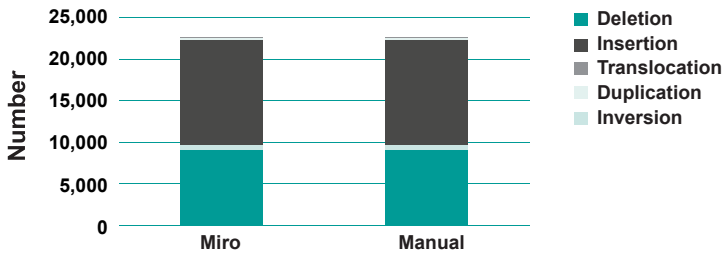
Figure 2: SMRTbell Prep Kit 3.0 library size distribution. Following automated preparation on the MIRO CANVAS, including bead-based size selection, libraries were examined with a Femto Pulse (Agilent) to demonstrate efficient removal of small library molecules.

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 (**Figure 3**) and structural variant detection (**Figure 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.

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Conclusion

- MIRO CANVAS is an advanced DMF platform that can be used to automate library preparation with the PacBio SMRTbell Prep Kit 3.0.
- When using the SMRTbell Prep Kit 3.0 on the MIRO CANVAS, the protocol is fully automated from post-shear clean-up to elution.
- 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.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	
Pacific Biosciences of California	102-141-700	SMRTbell® prep kit 3.0	https://www.pacb.com/wp-content/uploads/Insert-SMRTbell-prep-kit-3.0.pdf
Coriell Institute for Medical Research	NA24385 (HG002)	High molecular weight DNA	https://catalog.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA24385&Product=DNA

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