

# Automating PacBio® SMRTbell® whole genome sequencing library prep on the 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.<sup>1</sup> Additionally, long-read libraries that are prepared without PCR amplification avoid a common source of base composition bias in sequencing data.<sup>2</sup>

Many long-read library prep workflows have traditionally used 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 easily automated long-read library preparation.

The [MIRO CANVAS NGS prep system](#) 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.
- Using the SMRTbell Prep Kit 3.0 protocol, MIRO CANVAS total library quantities, peak sizes and primary sequencing metrics are indistinguishable from manually prepared libraries.

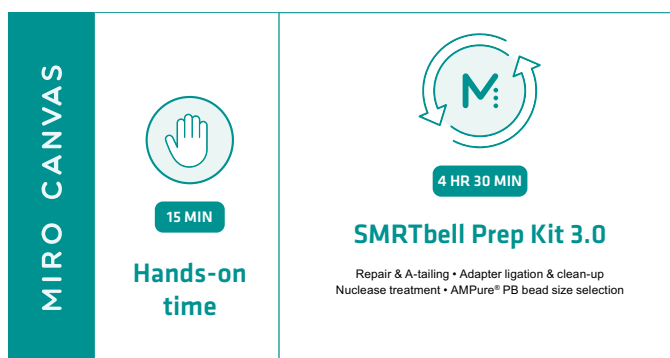
## Overview: How to automate PacBio SMRTbell WGS on the MIRO CANVAS

### MIRO CANVAS NGS PREP SYSTEM



## Experimental set-up

The SMRTbell Prep Kit 3.0 protocol was designed with automated systems – such as the MIRO CANVAS – in mind, and has 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 Q32850 Qubit™ dsDNA Quantification Assay Kit, Broad Range, or similar. Post-shearing clean-up, repair and A-tailing, adapter ligation, post-ligation clean-up, nuclease treatment and bead-based size selection for the SMRTbell Prep Kit 3.0 are all automated on the MIRO CANVAS (**Figure 1**), resulting in a ready-to-sequence library.



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

## Method and results

SMRTbell libraries were constructed with 1 µg of high quality, high molecular weight NA24385 (HG002)\* DNA, manually and using the MIRO CANVAS. Final library quantities were assessed using broad range or high sensitivity Qubit kits. The MIRO CANVAS produced comparable libraries to manual preparation for each of the kits evaluated (**Table 1**).

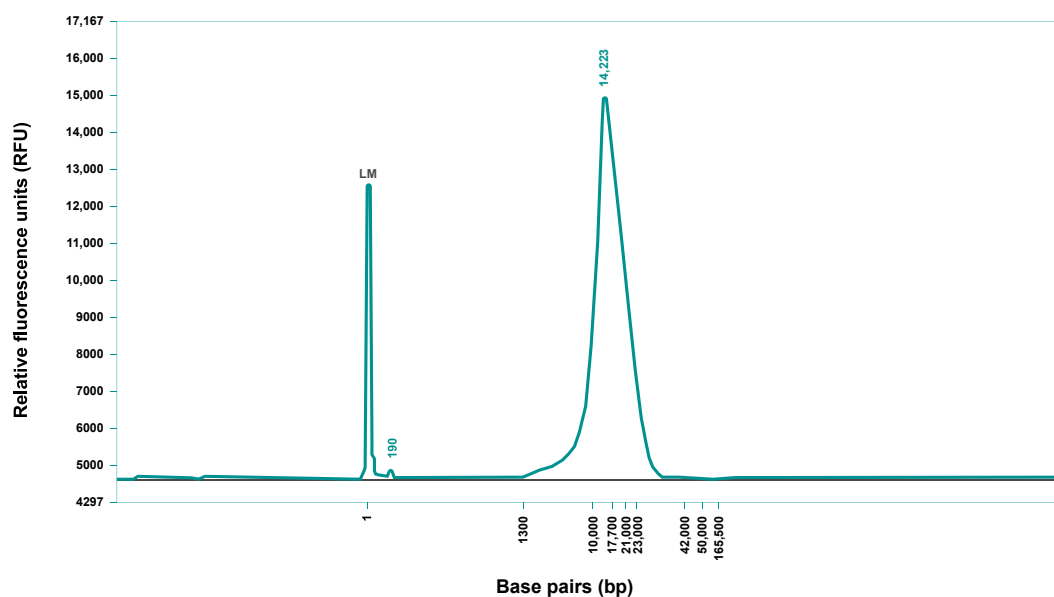
\* NA24385 DNA was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

**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).

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

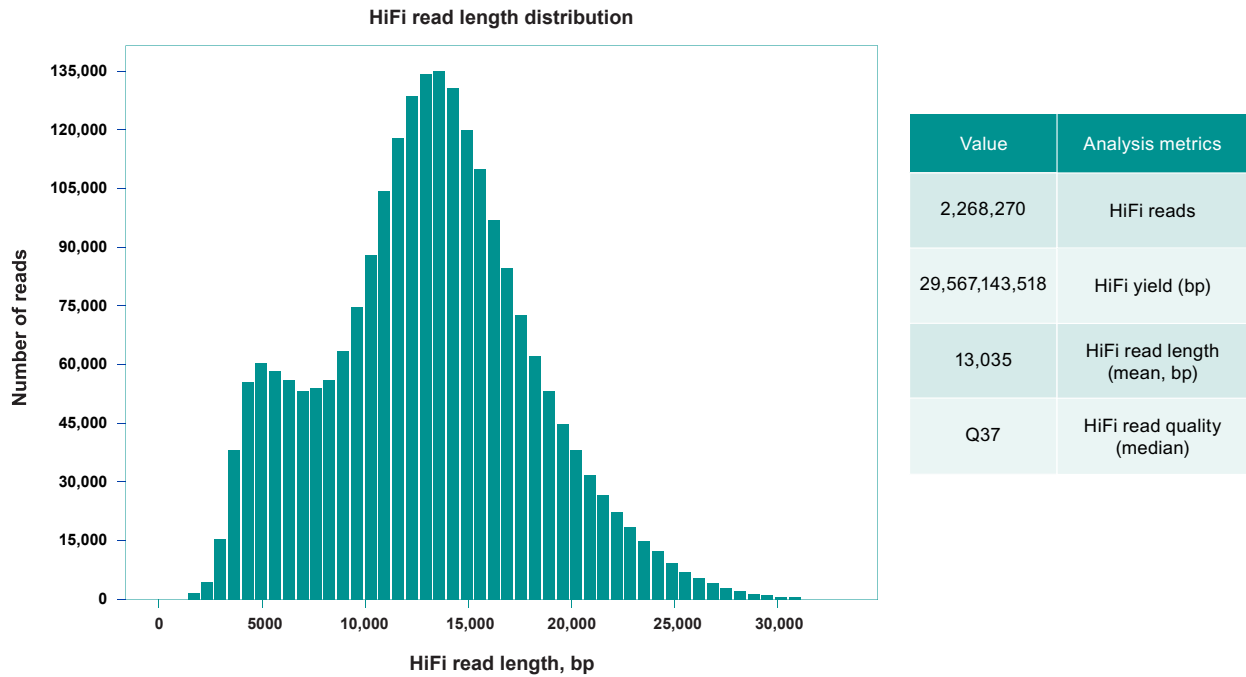
**Efficient removal of small molecules from libraries using diluted bead-based size selection with the SMRTbell Prep Kit 3.0**

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**).

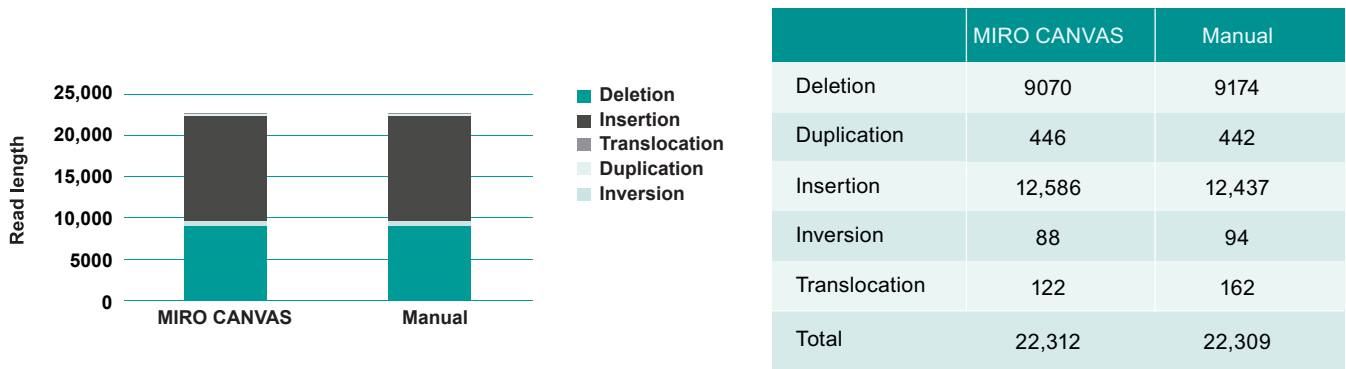


**Figure 2:** SMRTbell Prep Kit 3.0 library size distribution. Following automated preparation on the MIRO CANVAS, including bead-based size selection, libraries were examined with a Femto Pulse (Agilent) to demonstrate efficient removal of small library molecules. LM=lower marker.

SMRTbell libraries were sequenced on a Sequel<sup>®</sup> II System using binding kit 2.2, sequencing kit 2.0 (PacBio), 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**).



**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.



**Figure 4:** SMRTbell Prep Kit 3.0 structural variant detection. MIRO CANVAS detection of deletions, duplications, insertions, inversions and translocations in an NA24385 (HG002) DNA sample are all comparable to manually prepared libraries.

## Conclusion

- The 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 | <a href="https://www.integra-biosciences.com/en/ngs-automation/miro-canvas">https://www.integra-biosciences.com/en/ngs-automation/miro-canvas</a>   |
| INTEGRA Biosciences                    | M-02-0001-001-03 | MIRO Cartridge              | <a href="https://www.integra-biosciences.com/en/ngs-automation/miro-canvas">https://www.integra-biosciences.com/en/ngs-automation/miro-canvas</a>   |
| INTEGRA Biosciences                    | M-03-0001-001-01 | MIRO Dropgloss              | <a href="https://www.integra-biosciences.com/en/ngs-automation/miro-canvas">https://www.integra-biosciences.com/en/ngs-automation/miro-canvas</a>   |
| PacBio                                 | 102-141-700      | SMRTbell prep kit 3.0       | <a href="https://www.pacb.com/wp-content/uploads/Insert-SMRTbell-prep-kit-3.0.pdf">https://www.pacb.com/wp-content/uploads/Insert-SMRTbell-prep-kit-3.0.pdf</a>   |
| Coriell Institute for Medical Research | NA24385 (HG002)  | High molecular weight DNA   | <a href="https://catalog.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA24385&amp;Product=DNA">https://catalog.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA24385&amp;Product=DNA</a> |

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