MIROCULUS
SCIENCE SIMPLIFIED

Enabling the Illumina DNA PCR-Free Library Prep Kit on Miro Canvas®

Data from a demonstrated protocol

Key points

- **Library preparation with the Illumina DNA PCR-Free Prep kit is fully automated on Miro Canvas**
- **This protocol has been demonstrated on Miro Canvas using 50–500 ng DNA inputs**
- **Insert sizes, yields, and sequencing metrics of libraries prepared on Miro Canvas using this protocol are comparable to those of manually prepared libraries**
- **Automation on Miro Canvas reduces the amount of hands-on time required for library preparation by >60% when using this protocol**

Introduction

Since next generation sequencing (NGS) library preparation protocols that include PCR can introduce amplification-associated artifacts into the pool of DNA for sequencing, there is an increasing demand for PCR-free protocols.[2] The Illumina DNA PCR-Free Prep kit follows a PCR-free workflow and is being increasingly used in sensitive applications such as whole genome sequencing (WGS) because it is both flexible and easy to automate.[3] Its on-bead tagmentation step is especially important for reducing library preparation time and sample input requirements. These features are of great interest for clinical applications such as tumor evaluations and newborn diagnostics, and are also important for research uses.

Miro Canvas is a digital microfluidics (DMF) platform that allows custom low-throughput workflow automation for complex protocols such as NGS library preparation. The system is compatible with a wide range of reagents[4], 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 Illumina DNA PCR-Free Prep kit in a protocol developed for Miro Canvas. The resulting research use only libraries can then be sequenced using Illumina sequencing platforms.

Experimental workflow

The Illumina DNA PCR-Free Prep Protocol was designed for fully automated use on Miro Canvas and has been tested using high quality DNA inputs within the 50–500 ng range.[5] Before beginning, DNA should be quantified using a Broad-Range Qubit quantification kit or similar. Tagmentation, post-tagmentation cleanup, ligation, and library cleanup steps are all automated on Miro Canvas (Fig. 1).[2] When needed, downstream normalization and pooling require hands-on time.

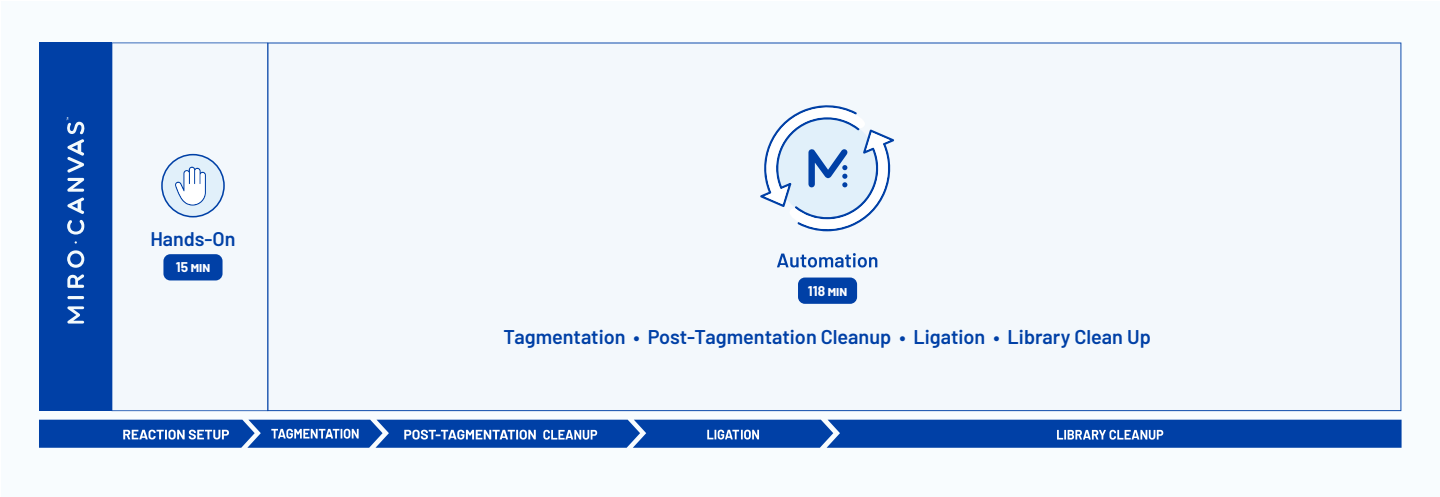


Figure 1. Experimental Workflow. The Miro Canvas automates all the steps following reaction setup, including: tagmentation, post-tagmentation cleanup, ligation and library cleanup.

Results

Automated workflow on Miro Canvas produces comparable library insert sizes, yields and metrics

The Illumina DNA PCR-Free Prep Protocol on Miro Canvas has been tested using 50, 300, and 500 ng of NA12878 gDNA*. After testing a range of bead:input DNA ratios using a single right-sided selection, a 1.6x ratio was determined to be optimal for obtaining libraries of an ideal size and with sufficient quantity for sequencing (Fig. 2). Shallow sequencing of libraries prepared with 300 ng of input gDNA on a MiSeq Micro PE150 run confirmed that the 1.6x ratio also resulted in the expected insert size of ~425 bp. Library insert sizes and yields were comparable between manual preparation and the automated workflow on Miro Canvas (Fig. 2).

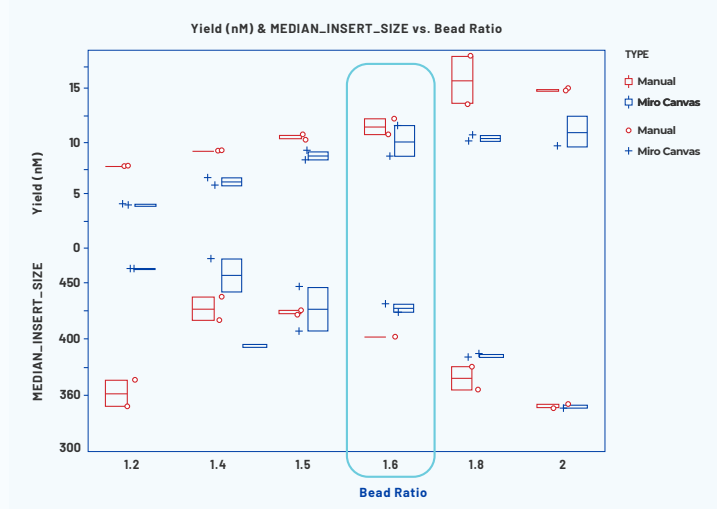


Figure 2. Library insert sizes and yields generated from different bead ratios when using 300 ng of input DNA.

Boxes show minimum, median, and maximum values.

*NA12878 DNA samples were obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research.

The 1.6x bead ratio also resulted in the lowest percentage of aligned bases being excluded due to all filters or due to overlapping reads, with values being comparable between manually prepared libraries and those generated using the automated workflow on Miro Canvas (Fig. 3).

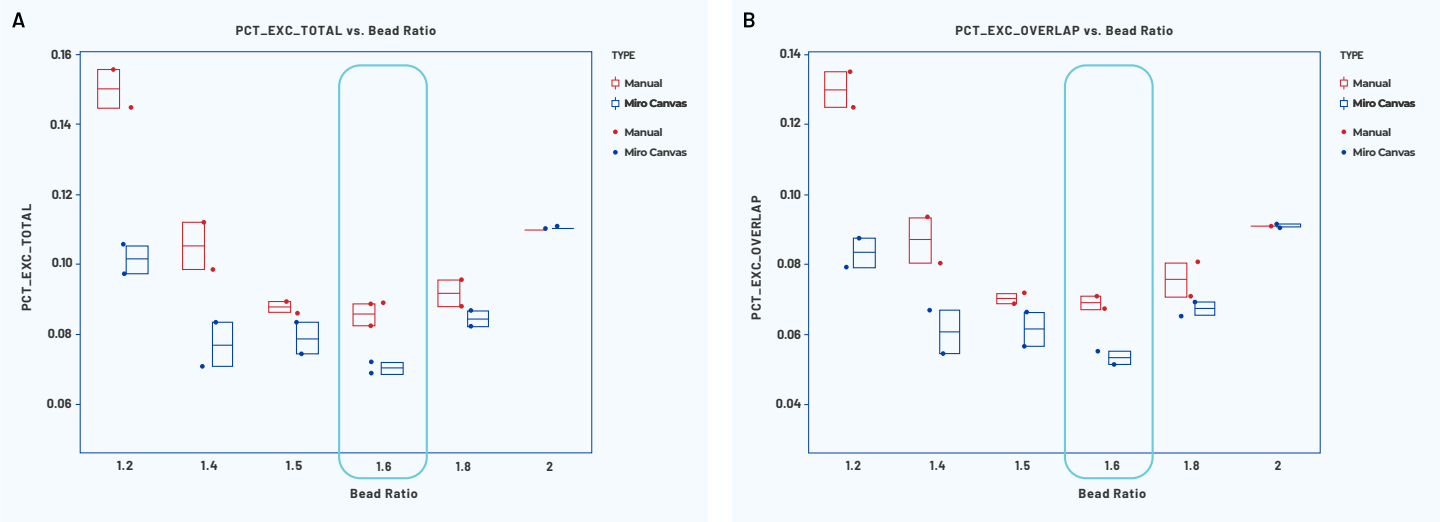


Figure 3. Base exclusion metrics for different bead ratios when using 300 ng of input DNA.

A. The total fraction of aligned bases excluded due to all filters. B. The fraction of aligned bases that were filtered out because they were the second observation from an insert with overlapping reads.

Boxes show minimum, median, and maximum values.

Sequencing shows comparable coverage depth of libraries

MiSeq coverage depth of libraries prepared manually with the Illumina DNA PCR-Free Prep kit were compared to coverage depth of libraries obtained by automation on Miro Canvas. The two distinct methods showed a high correlation of the percentage of bases covered at ≥1x over passed filter reads (Fig. 4).

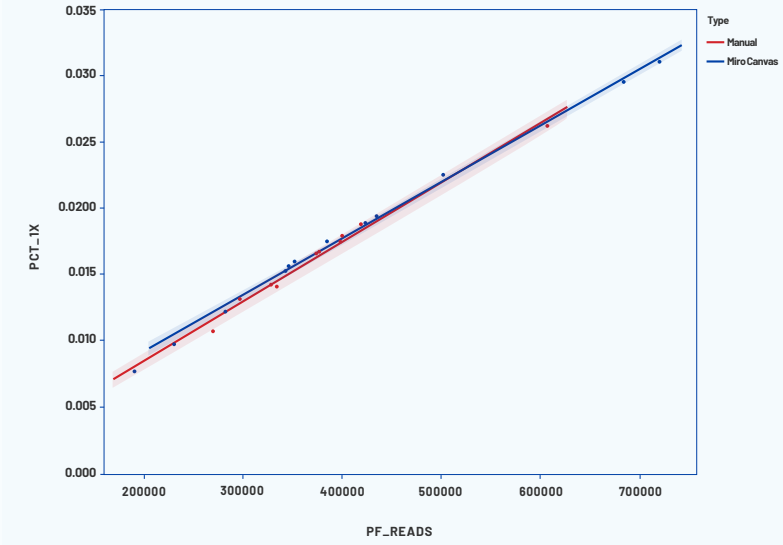


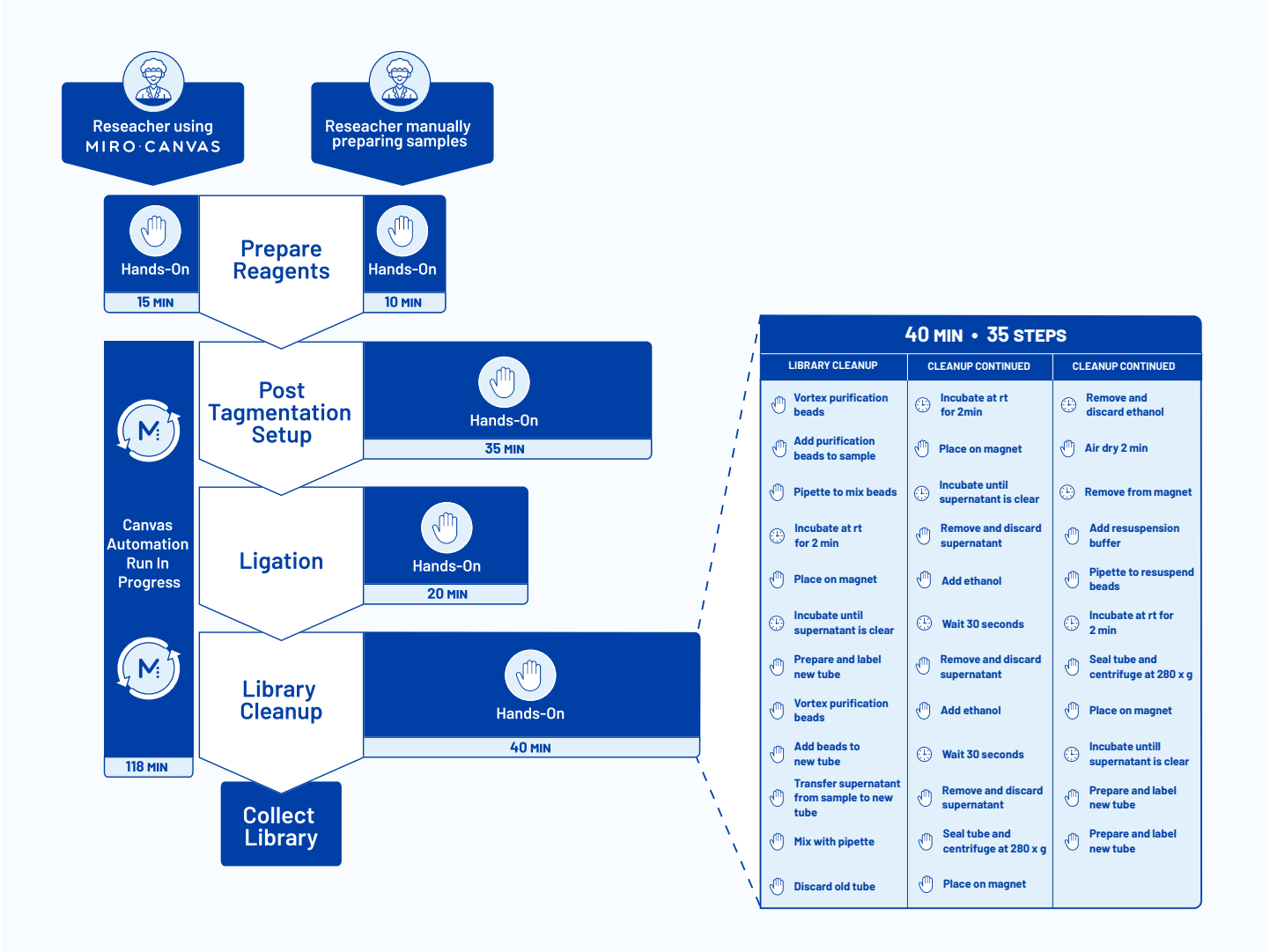
Figure 4. Percentage of bases at ≥1x coverage by against the number of reads passing Illumina's filter.

Sequencer: Illumina MiSeq® System.

Miro Canvas walk away automation reduces hands-on time

Although the total time required for library preparation with the Illumina DNA PCR-Free Prep kit is 23 minutes greater when automating on Miro Canvas, hands-on time is considerably less than for manual preparation (Table 1). Automation with Miro Canvas reduces hands-on time to zero for the tagmentation, post-tagmentation cleanup, ligation, and library cleanup steps.

Table 1. Average time requirements when manually preparing libraries or automating library preparation on Miro Canvas with the Illumina DNA PCR-Free Prep kit



Summary

Miro Canvas is an advanced DMF platform that can be used to automate library preparation with the Illumina DNA PCR-Free Prep kit. When using the Illumina DNA PCR-Free Prep Protocol for Miro Canvas, the protocol is fully automated from the tagmentation incubation step to elution, and can be used with DNA inputs ranging from 50 to 500 ng. Both Miro Canvas and manual library preparation yield comparable results, but the true walk away automation and minimal hands-on time provided by Miro Canvas make it a valuable addition to any laboratory.

References

1. Kebschull JM *et al. Nucleic Acids Res* 2015; 43 (21): e143.
2. Yoo J et al. Poster 32 presented at the Association of Biomolecular Resources Facilities (ABRF) 2021 Virtual Annual Meeting; 7–11 March 2021.
3. Illumina DNA PCR-Free Prep. Available at: https://www.illumina.com/products/by-type/sequencing-kits/library- prep-kits/ dna-pcr-free-prep.html. Accessed April 2021.
4. Miroculus. New Class of Technology. Available at: https://miroculus.com/technology/. Accessed April 2021.
5. Miroculus, Inc. protocol; 2021. Available at: https://miroculus.com.
6. Illumina, Inc. Illumina DNA PCR-Free Library Prep, Tagmentation Reference Guide. Document 1000000086922 v03; February 2021.

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Revision History

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Revisions
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