Application Note

INTEGRA

Enabling the Illumina DNA PCR-Free Library Prep kit on the MIRO CANVAS® NGS prep system

Introduction

There is an increasing demand for next generation sequencing (NGS) library preparation protocols that do not include PCR to avoid the introduction of PCR bias into the pool of DNA for sequencing.[1]

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.[2]

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.[3]

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 platforms.

Key benefits

- 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.
- 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.

Overview: How to enable the Illumina DNA PCR-Free Library Prep kit on MIRO CANVAS

MIRO CANVAS NGS Prep System

Experimental set-up

The Illumina DNA PCR free library 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. DNA should be quantified using a Broad-Range Qubit quantification kit (or similar) before beginning the protocol. Libraries are quantified using ssDNA Qubit Quantification kit or qPCR. Tagmentation, post-tagmentation cleanup, ligation and library cleanup steps are all automated on MIRO CANVAS (**Figure 1**). Downstream normalization and pooling require hands-on time.

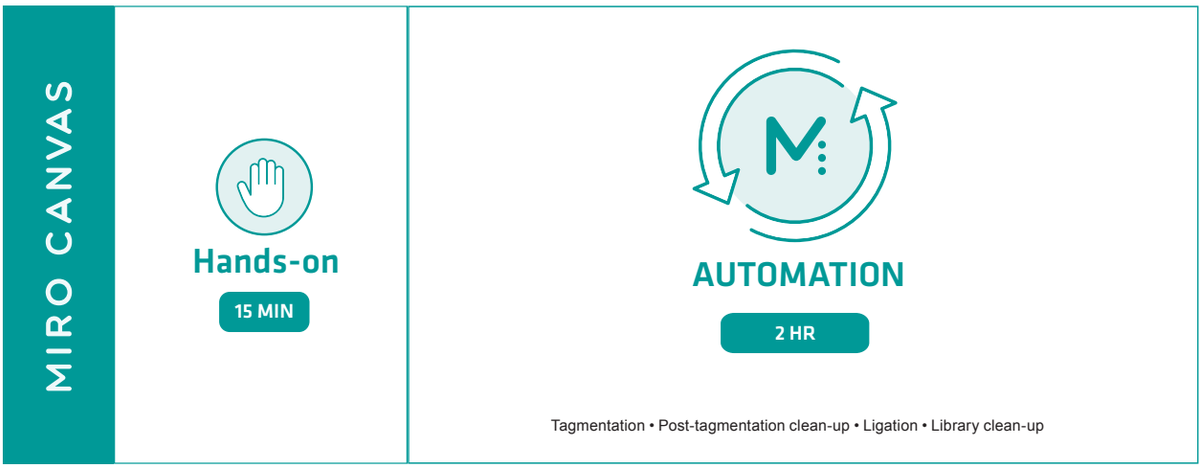

Figure 1: Experimental set-up. The MIRO CANVAS automates all the steps following reaction set-up, including tagmentation, post-tagmentation clean-up, ligation and library clean-up.

Results

Automating the experimental workflow on MIRO CANVAS produces library yields and insert sizes that result in quality sequencing metrics. The Illumina DNA PCR-Free Prep protocol for standard input has been modified and tested using 50-500 ng NA12878 gDNA* on MIRO CANVAS. In this modified version (**Table 1**), combining the standard input protocol volumes of DNA input and bead-linked transposomes PCR-free (BLT-PF) with low input single-sided bead purification (1.8x ratio) was determined to be optimal for obtaining libraries of an ideal size and with sufficient quantity for sequencing (**Table 2**). Libraries prepared with as low as 50 ng of input gDNA were sequenced on a NovaSeq S4 PE150. The 1.8x ratio resulted in the kit's expected insert size of ~450 bp for >300 ng input. BLT-PF and DNA input volumes will need to be further adjusted for 50 ng input to achieve the expected insert size in both manual preparation and the automated workflow on MIRO CANVAS.

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

Table 1: Conditions for sample purification bead addition and insert size selection across different inputs of DNA tested.

	DNA VOLUME INPUT	BLT VOLUME INPUT	VOLUME INTO FIRST CLEAN-UP	FOLD FIRST CLEAN-UP	VOLUME FIRST IPB	TOTAL VOLUME INTO SECOND CLEAN-UP	FOLD SECOND CLEAN-UP	VOLUME SECOND IPB
Standard input	25 µl	15 µl	45 µl	0.8	36 µl	76 µl	1.8	42 µl
Low input	30 µl	10 µl	45 µl	1.8	81 µl	N/A	N/A	N/A
Modified version	25 µl	15 µl	45 µl	1.8	81 µl	N/A	N/A	N/A

Table 2: Library insert sizes and yields generated from different inputs of unsheared NA12878 DNA.

	MANUAL			MIRO CANVAS		
TOTAL WORKFLOW TIME	1 HR 45 MIN			1 HR 55 MIN		
DNA input amount (ng)	500	300	50	500	300	50
Mean yield (nM)	34.4	26.7	5.3	26.2	17.8	5.3
Median insert size (bp)	496	475	364	454	447	285

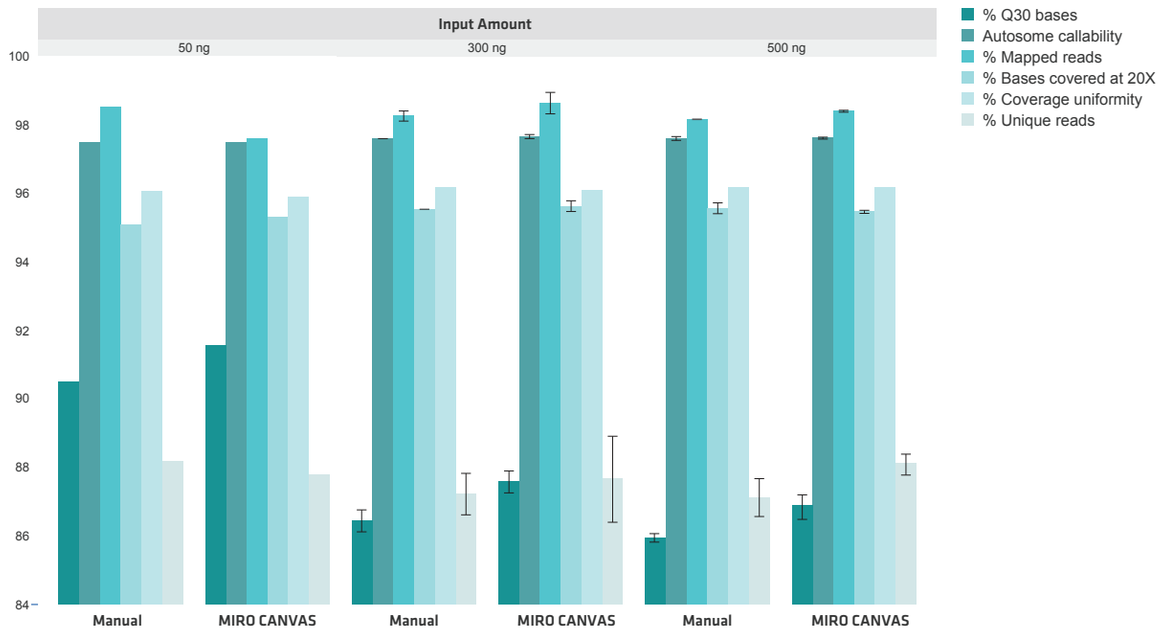

Figure 2: Sequencing metrics performance across a range of input DNA.

Illumina DNA PCR-free libraries prepared from a range of DNA inputs using either manual or MIRO CANVAS preparation methods demonstrate comparable % Q30 score, autosome callability, % mapped reads, % of bases covered at 20X, coverage uniformity and % unique reads.

The resulting sequencing metrics values are comparable between manually prepared libraries and those generated using the automated workflow on MIRO CANVAS (**Figure 2**). For DNA input amounts >300 ng, MIRO CANVAS libraries match or exceed the sequencing metrics for manual libraries including base call accuracy, passing genotype calls in autosomal chromosomes, reads that confidently map to the reference genome, the % of bases covered at 20X, uniformity of coverage and duplication rates. QC metrics used in applications aimed at variant detection were additionally examined after subsampling to 40X sequencing coverage (**Table 3**). MIRO CANVAS libraries presented equal or better F1 scores for both SNVs and INDELs, as well as % bases covered at 20X across all input ranges of tested DNA input.

Table 3: The performance of QC metrics relevant for variant calling across a range of input DNA. All samples were subsampled to 40X sequencing coverage.

SAMPLE ID	SEQUENCED BASES (GB)	AVERAGE AUTOSOMAL COVERAGE	% BASES COVERED AT 20x	AVERAGE MITOCHONDRIAL COVERAGE	TOTAL NUMBER SNVs	SNVs F1 SCORE	TOTAL NUMBER INDELs	INDELs F1 SCORE
Manual 500 ng	127	35.29	94.75	7527.37	4,052,452	99.90 %	25,511	99.55 %
MIRO CANVAS 500 ng	126	35.54	94.86	6023.38	4,055,818	99.90 %	25,565	99.59 %
Manual 300 ng	127	35.31	94.87	6667.97	4,051,961	99.90 %	25,473	99.57 %
MIRO CANVAS 300 ng	127	36.21	95.03	6947.82	4,054,773	99.91 %	25,563	99.59 %
Manual 50 ng	122	34.34	94.18	9902.86	4,053,721	99.88 %	25,772	99.50 %
MIRO CANVAS 50 ng	123	34.68	94.56	7956.68	4,054,906	99.89 %	25,643	99.54 %

MIRO CANVAS walk away automation reduces hands-on time

The total time required for library preparation with the Illumina DNA PCR-Free Prep kit is 25 minutes greater when automating on MIRO CANVAS, but the hands-on time is considerably less than for manual preparation workflows (**Table 1**). Automation with MIRO CANVAS reduces hands-on time to zero for the tagmentation, post-tagmentation cleanup, ligation, and library cleanup steps.

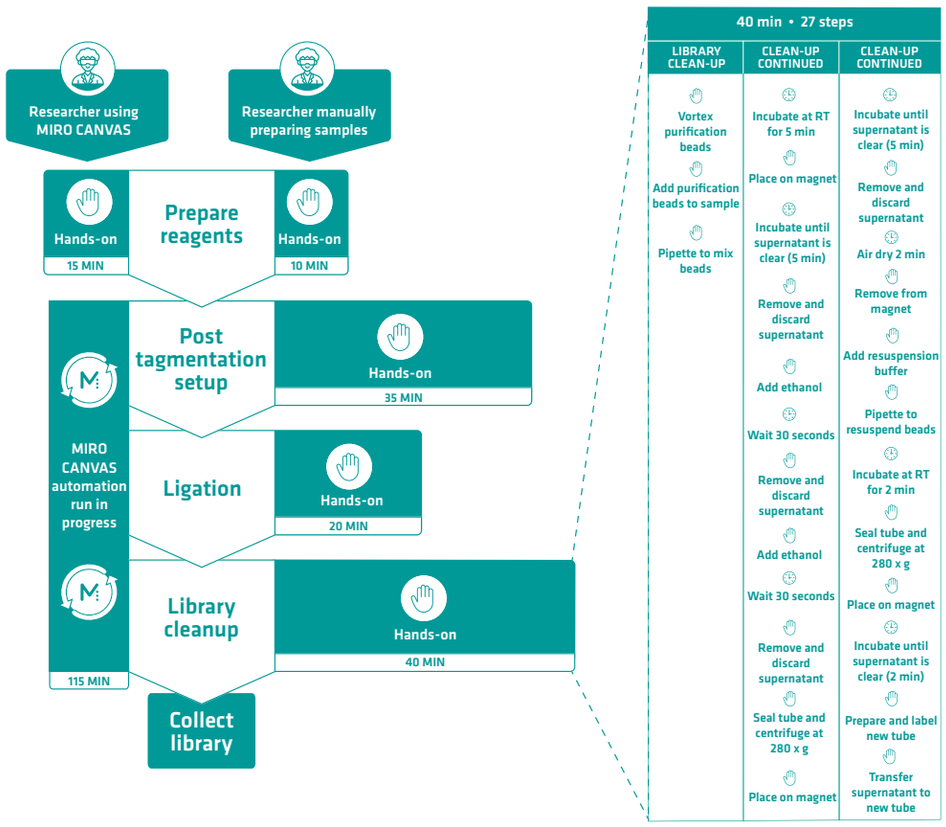

Figure 3: Average time requirements when manually preparing libraries or automating library preparation on MIRO CANVAS with the Illumina DNA PCR-Free Prep kit.

Conclusion

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 library 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 50-500 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 makes 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.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
Illumina	20041794	Illumina DNA PCR free kit	https://www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html
Coriell Institute for Medical Research	NA12878	DNA standard	https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA12878

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