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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**
- **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 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 >300ng input. BLT-PF and DNA input volumes will need to be further adjusted for 50ng input to achieve the expected insert size in both manual preparation and the automated workflow on Miro Canvas.

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

	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	25µL	15uL	45µL	0.8	36µL	76µL	1.8	42µL
Low input	30µL	10µL	45µL	1.8	81µL	N/A	N/A	N/A
Modified version	25µL	15µL	45µL	1.8	81µL	N/A	N/A	N/A

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

	Manual			Miro Canvas		
Total workflow time	**1 hr 45 min**			**2 hrs 13 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**

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

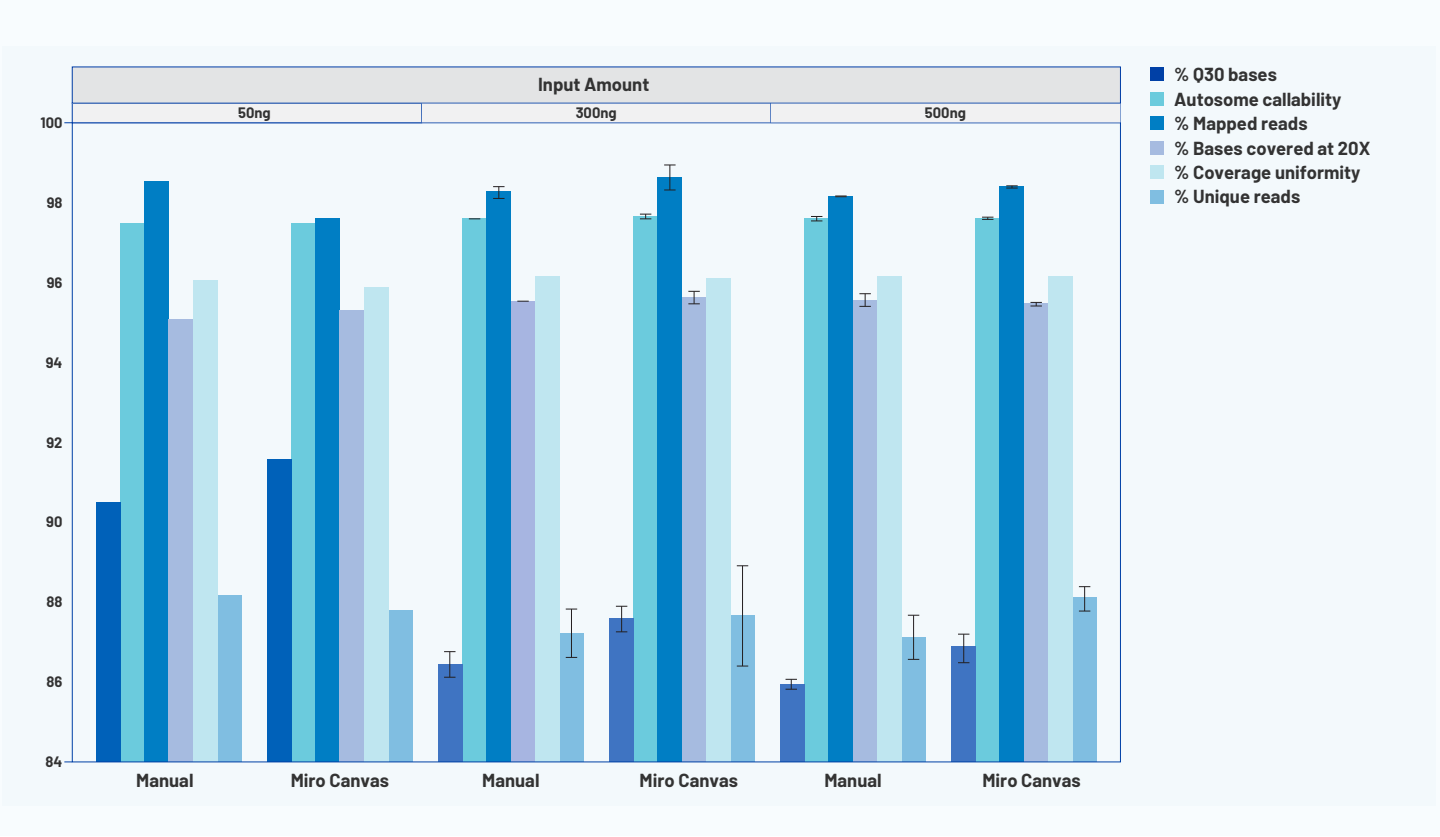


Figure 2. Sequencing metrics performance across a range of input DNA.

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 (Fig. 2). For DNA input amounts >300ng, Miro Canvas libraries match or exceed manual libraries sequencing metrics when considering base call accuracy, passing genotype calls in autosomal chromosomes, reads that confidently map to the reference genome, the percentage of bases covered at 20X, uniformity of coverage and duplication rates. QC metrics with relevance in applications where variant detection is the goal were additionally examined (Table 3). Coverage and the total number of SNVs and INDELs present similar values between manually prepared and Miro Canvas libraries.

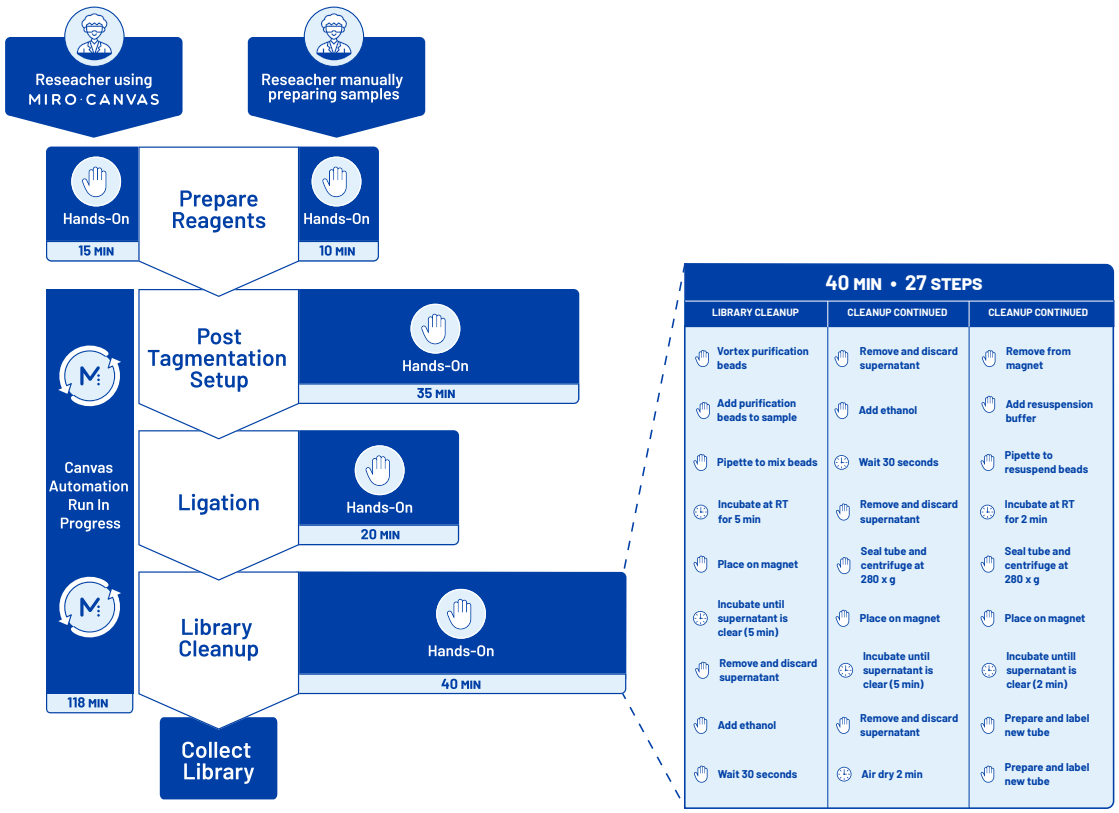
Sample ID	Sequenced bases (Gb)	Average Autosomal coverage	Average Mitochondrial coverage	Total # SNVs	Total # INDELs (Het)
Manual 500 - 1	200	54.7	10,486.50	4,037,266	25,941
Manual 500 - 2	232	62.83	10,604.94	4,040,675	25,841
Miro 500 - 1	197	54.43	8,414.38	4,038,031	25,921
Miro 500 –2	207	56.93	8,546.22	4,040,038	25,916
Manual 300 - 1	214	58.62	9,984.20	4,038,580	25,925
Manual 300 - 2	215	58.44	9,809.56	4,037,061	25,943
Miro 300 - 1	203	56.88	9,738.08	4,037,220	25,953
Miro 300 - 1	250	62.21	11,474.07	4,043,497	25,951
Manual 50 - 1	172	48.04	9,902.88	4,027,371	26,088
Miro 50 - 1	211	58.02	10,822	4,031,413	26,153

Table 3. QC metrics performance relevant for variant calling across a range of input DNA.

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 28 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.

Figure 3. 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

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Revision History

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Revisions
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