The Element AVITI™ System and the Miro Canvas™ digital microfluidic platform combine for a high performance, NGS cost saving solution

Data from an enabled protocol

Key points

- PCR-Free libraries prepared on Miro Canvas were sequenced on the Element AVITI System, conveniently taking you from sample to sequencing data using only two benchtop platforms
- Previously demonstrated Miro PCR-Free Whole Genome Sequencing library prep protocol used to generate PCR-free libraries with 100 ng of mechanically sheared DNA input
- Sequencing metrics of libraries prepared on Miro Canvas demonstrate highly sensitive and specific SNP/indel detection
- Miro Canvas reduces the hands on time needed to prepare samples for sequencing using the AVITI platform by 33%
- The low operating costs of Miro Canvas and AVITI platforms combine to make sequencing significantly more accessible to researchers

Introduction

Next generation sequencing (NGS) technology has been evolving rapidly over the past decade. Advances have been made across a plethora of scientific applications that require NGS but also in novel sequencing approaches that are becoming available, such as the Element AVITI System[1]. Many of these advances show great potential to reduce the cost of sequencing in time, dollars, and space. Despite the advancements in NGS technologies and applications, researchers still must consider technician time, laboratory space, sample processing turn around time, assay complexity, and capital expenditures for automation instruments. Balancing these considerations without sacrificing high quality sequencing data is not trivial.

Miro Canvas is a digital microfluidics (DMF) platform that allows workflow automation for complex NGS library preparation protocols, and is compatible with a wide range of reagents[2]. This low throughput instrument, weighing in at just 6 kg, allows for quick sample processing with truly walkaway protocols that free up technical resources. Similarly, the Element AVITI System is designed to work seamlessly in any NGS workflow. It features scalable output of up to 800M+ reads on each of its two random access flow cells for workflow flexibility, data quality of >90% Q30 2x150bp for best in class sequencing accuracy, and factory level operating cost savings without factory scale batch requirements.

This application note describes the results that can be expected when using the Miro PCR-Free Whole Genome Sequencing Library Prep Protocol developed for Miro Canvas. The resulting research use only libraries can then be sequenced using the Element Biosciences AVITI sequencing platform. Combining Miroculus and Element Biosciences technologies can offer a simple and streamlined workflow for laboratories that care about performance, cost and flexibility in NGS sample preparation and sequencing.

Experimental workflow

The Miro PCR-Free Whole Genome Sequencing Library Prep Protocol has been validated for high-quality, mechanically sheared human gDNA within the 100–500 ng input range. NA12878 gDNA gets sheared to an average size of 360–450 bp using the Covaris® M220 instrument with the following settings: peak power, 50; duty factor, 20; cycles/burst, 200. DNA shear time is optimized for each individual batch. DNA quantification is carried out after DNA shearing and is performed using the Qubit™ dsDNA BR Assay Kit or the High-Sensitivity DNA Kit from Agilent Technologies, Inc. together with the Bioanalyzer instrument (sample dilution may be required). Post shear bead cleanup, end repair and A-tailing, adapter ligation and two back to back bead cleanup steps are all automated on Miro Canvas (Figure 1). The concentration of product libraries is measured using the KAPA Library quantification kit (Roche product number: 07960255001). Following linear library preparation, the Element Adept Library Compatibility kit (Element product number: 830-00003) circularizes libraries for sequencing on the AVITI System (Element product number: 880-00001). The Adept workflow is an easy 75-minute procedure with only 25-minutes of hands-on time and does not use PCR amplification[3,4] (Figure 1).

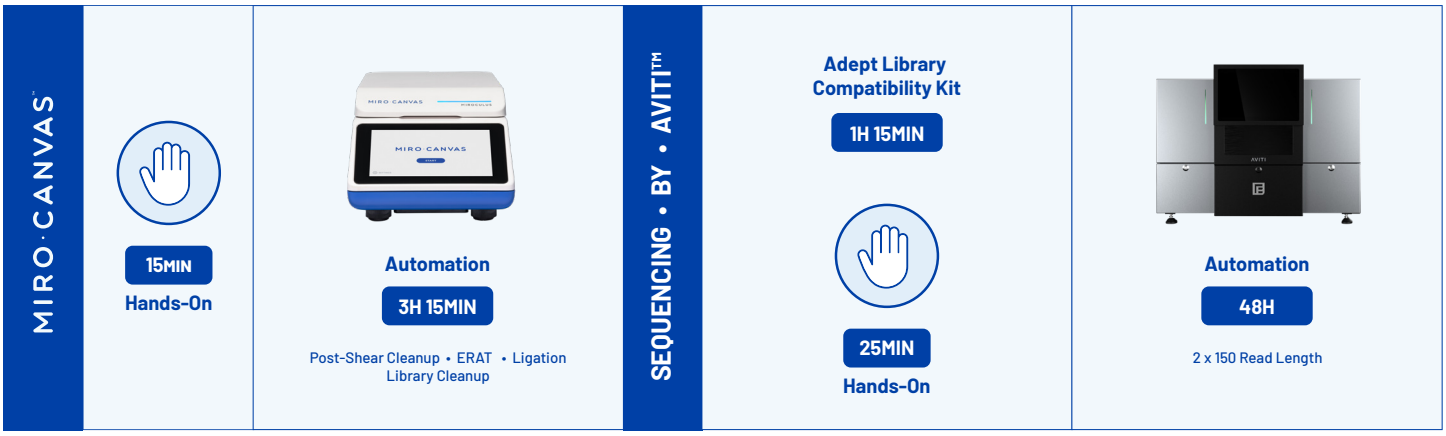

Figure 1. Experimental Workflow. The Miro Canvas automates all the PCR-Free Whole Genome Sequencing Library Prep steps following reaction setup, including: Post shear bead cleanup, end repair and A-tailing, adapter ligation and two back to back bead cleanups. Downstream library circularization using the Adept Library Compatibility Kit was manually performed.

Results

Automated workflow on Miro Canvas produces library yields and insert sizes able to generate high quality, PCR-Free sequencing data

Using 100 ng of Covaris sheared human NA12878* gDNA per sample, five replicate Miro PCR-Free Whole Genome Sequencing Libraries were prepared using conventional, manual linear library preparation, and an equivalent number of replicate libraries were prepared in parallel with five Miro Canvas instruments. Resulting product libraries yields were measured by qPCR quantification (Figure 2), and all indexed libraries had enough yield to be combined into a single reaction for manual, downstream circularization steps with the Adept Library Compatibility kit. The circularized manual and Miro Canvas prepared library pool was then sequenced on the AVITI System at 2x150bp Read Length. To obtain >35x coverage per sample, the resulting data from each of the 5 replicates was combined and then down sampled to 35X coverage (~360M 2X150 reads). Down sampled FASTQs were then aligned to hg38 using Sentieon BWA. Variant calling was performed with Sentieon DNAscope (using the AVITI specific model) and benchmarked against the NIST v4.2.1 truth sets using hap.py. Miro Canvas libraries insert sizes were found to be similar to the manual preps (Figure 2). The coverage distribution also appeared to be uniform and similar between the two sample preparations, centered at ~35x (Figure 3). Also, the cumulative coverage plot indicates that >92.5% of bases have 20X or more coverage (Figure 3).

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

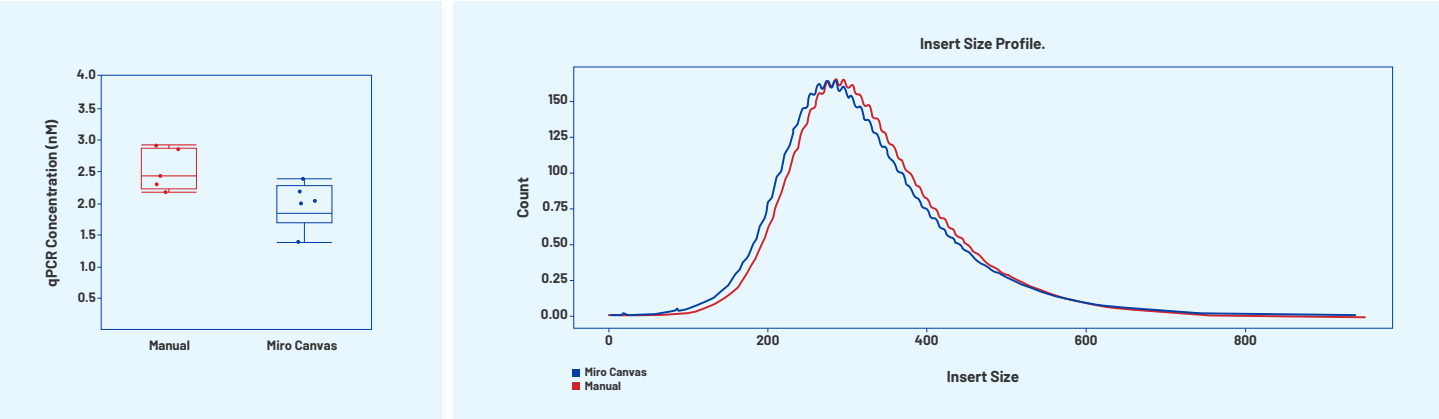

Figure 2. Product PCR-Free libraries concentration (nM) and insert sizes from 100ng sheared input NA12878 gDNA. Miro Canvas library insert size in blue. Manual library insert size in red.

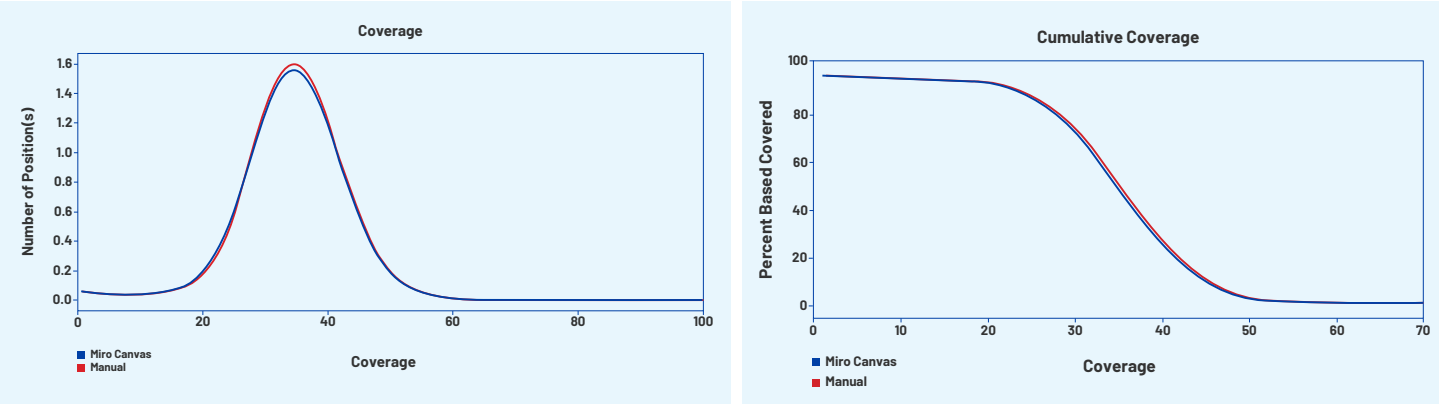

Figure 3. Coverage distribution and cumulative coverage after downsampling and alignment. Miro Canvas library insert size in blue. Manual library insert size in red.

The AVITI sequencer was able to generate near-unbiased coverage across GC content of both Miro Canvas and manually prepared sequenced libraries. No coverage bias was seen in the range of %GC content where adequate data was available (Figure 4).

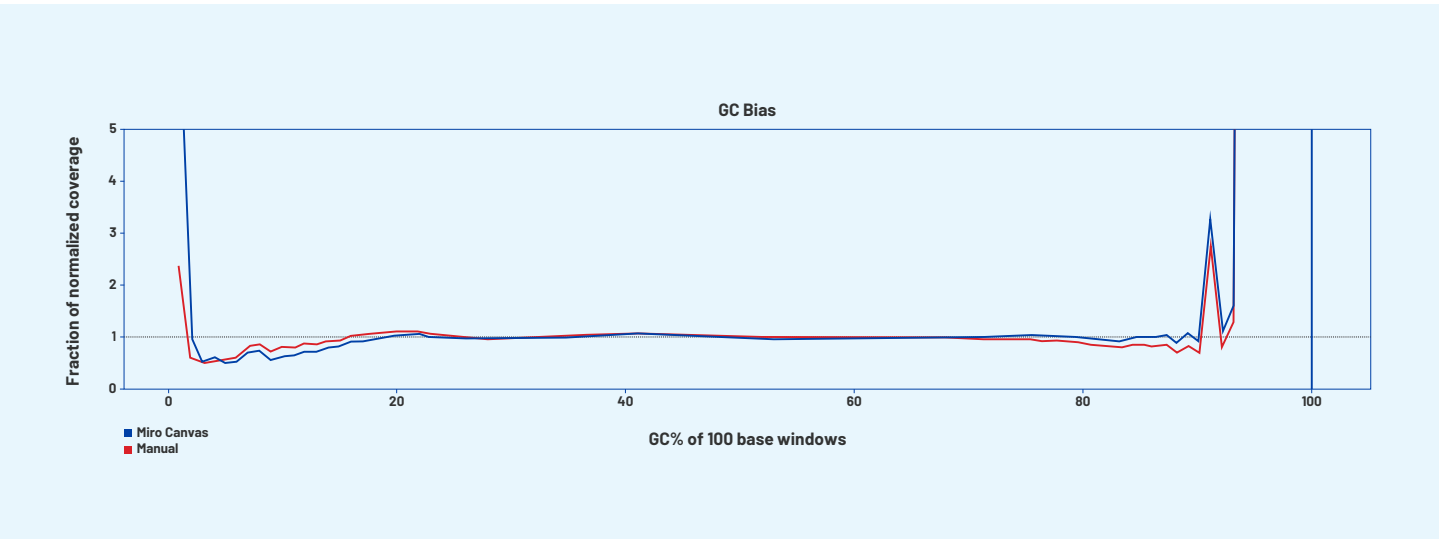

Figure 4. GC Bias curve. Miro Canvas library insert size in blue. Manual library insert size in red.

Miro Canvas libraries presented comparable sequencing metrics when considering base call accuracy, % reads aligned, % of bases covered at 20X and % duplicate reads (Table 1). QC metrics with relevance in applications where variant detection is the goal were additionally examined (Table 1). F1 scores for both SNPs and INDELs showed similar values between manually prepared and Miro Canvas libraries, allowing delivery of confident variant calling.

Sample ID	#Reads (2x150)	% PF Reads Aligned	% Bases ≥ 20x	% Duplicates	% Q30	SNP F1	INDEL F1
Manual	360,323,090	99.60%	92.79%	0.60%	90.45%	0.9960	0.9962
Miro Canvas	360,321,396	99.12%	92.62%	0.61%	90.11%	0.9958	0.9958

Table 1. Sequencing metrics performance from PCR-Free libraries constructed using 100 ng sheared input NA12878 gDNA. Base coverage was calculated using samtools stats across the entire hg38 reference not including N bases. SNP F1 and INDEL F1 are shown across all regions in the NIST v4.2.1 truth set for HG001.

Miro Canvas walk away automation reduces hands-on time

The Miro PCR-Free Whole Genome Sequencing Library Prep Protocol duration is 3 hours and 15 minutes. This is similar to the time needed to prepare libraries manually. However, with Miro Canvas hands-on time is considerably less than for manual preparation (Figure 1). Post shear bead cleanup, end repair and A-tailing, adapter ligation and two back to back bead cleanup steps are all automated on Miro Canvas, therefore not requiring frequent visits to the bench to execute the protocol's hands-on steps. Miro Canvas generated libraries convert seamlessly in the manual Adept Library Compatibility workflow for sequencing on the AVITI system. Miro Canvas automation of the Adept Library Compatibility kit could help further streamline and fully automate end to end sample preparation for the Element AVITI System.

Summary

Miro Canvas is an advanced Digital Microfluidics platform that can be used to automate PCR-Free library preparation for the Element AVITI System and yield very high-quality results. The protocol is fully automated from the post shear bead cleanup to product library elution and can be used with DNA inputs as low as 100 ng. Both Miro Canvas and manual library preparation yield comparable AVITI sequencing 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. Slatko BE et al. Curr Protoc Mol Biol 2018; 122 (1): e59.
2. Miroculus. New Class of Technology. Available at: https://miroculus.com/technology/. Accessed April 2021.
3. Element Adept Library Compatibility Workflow Guide (MA-00001)
4. Element AVITI System Workflow Guide (MA-00008)

Legal Disclaimers

Revision History

Version
1.0

Revisions
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