

DNA Inputs for PCR-Free Whole Genome Sequencing (WGS) Library Preparation on Miro Canvas™

Maximizing the flexibility of human DNA sample inputs

KEY POINTS

- Miro Canvas is a digital microfluidics platform that enables automated workflows for next generation sequencing library preparation
- Library preparation with Miro Canvas has been validated for sheared human gDNA inputs within the 100–500 ng range using the Miro PCR-Free Whole Genome Sequencing Library Prep Protocol
- Libraries prepared with Miro Canvas have comparable yields to those obtained with manual preparation methods across a range of DNA inputs, and require less hands-on time
- Compared to libraries prepared using manual preparation methods, libraries prepared with Miro Canvas yield high-quality sequencing results with improved genomic coverage

Introduction

Next generation sequencing (NGS) technology, which has been rapidly evolving over the past decade, can be used in a plethora of scientific applications.¹ While advancements have been made in all steps of the NGS workflow, library preparation continues to be the main bottleneck.² Library preparation typically involves complex protocols that are associated with high costs (both in terms of time and resources) and inherent contamination.^{2,3}

Miro Canvas is a digital microfluidics (DMF) platform that delivers custom low-throughput workflow automation for complex protocols such as NGS library preparation. Simplifying and automating NGS library preparation with Miro Canvas and its one-time use, disposable cartridges not only minimizes hands-on time but also yields high-quality libraries that are associated with improved sequencing coverage.

This technical note provides an overview of the results that can be obtained when using Miro Canvas for human whole genome sequencing (WGS) library preparation and explores what can be expected when using the existing Miro PCR-Free Whole Genome Sequencing Library Prep Protocol with DNA inputs outside of the validated range.⁴

Miro Canvas For WGS Library Preparation

VALIDATED INPUT RANGE

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. At Miroculus, NA12878 gDNA is 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.⁴

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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).

The typical library yields that can be obtained when using the Miro PCR-Free Whole Genome Sequencing Library Prep Protocol with DNA inputs within the validated range are shown in Table 1. This table should be used as a guide for selecting appropriate DNA inputs based on the specific library requirements of the sequencer to be used.

Table 1. Expected average library preparation yields for Miro Canvas as a function of NA12878 DNA input

Sheared DNA Input* (ng)	Expected Library Yield† (nM)
100	1
150	2
200	3
500	6

*DNA input quantity is measured after shearing.

†As library yields can be impacted by a number of factors including input material quality, yields could be higher or lower than those shown in the table.

Sequencing Results

WGS libraries prepared with Miro Canvas yield sequencing results that are comparable to the results obtained with WGS libraries prepared manually or using 96-well automation. Furthermore, data indicate that less sequencing is required to reach 20x coverage for 95% of bases using libraries prepared with Miro Canvas (Fig. 1).

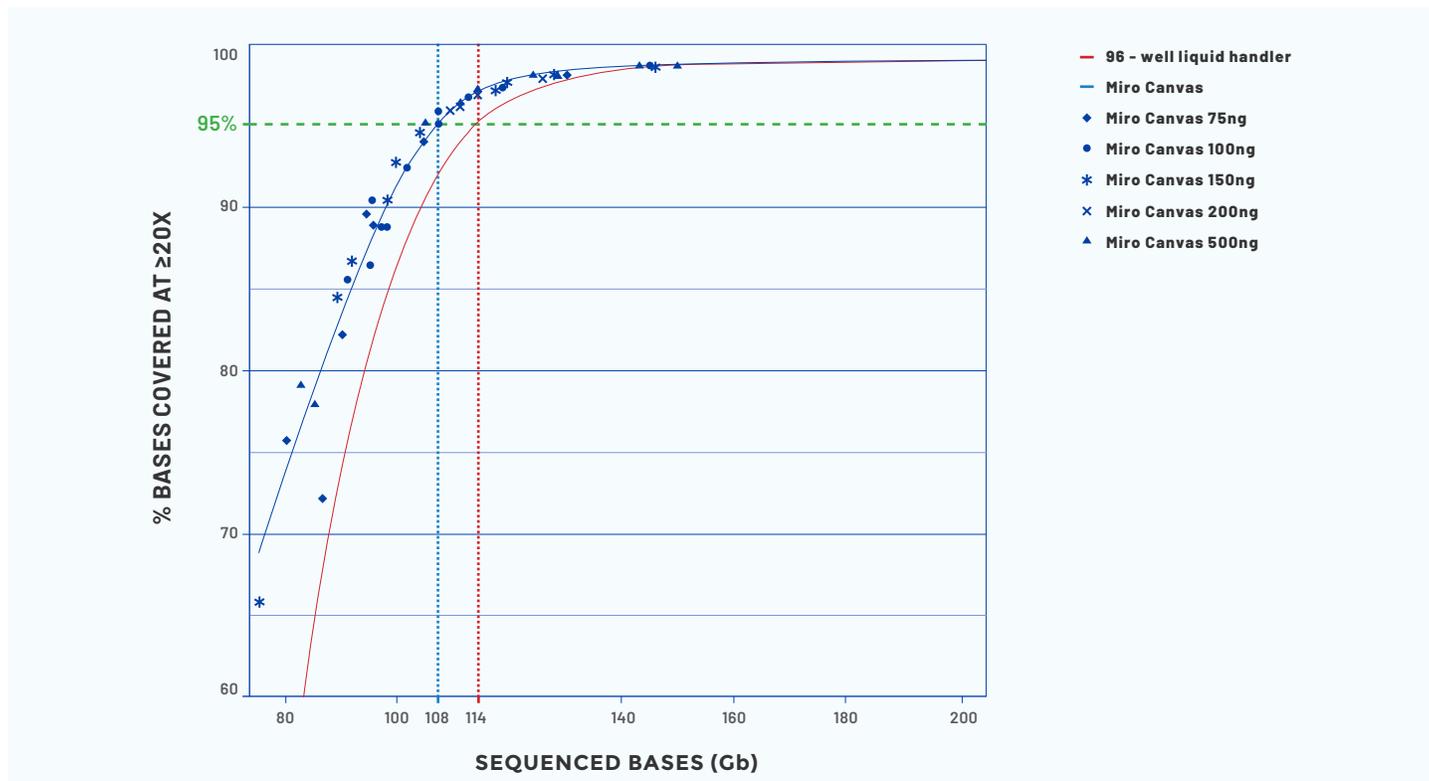


FIGURE 1. Percentage of bases at $\geq 20x$ coverage by total sequenced bases (Sequencer: Illumina NovaSeq S4 flow cell, 24 pooled WGS libraries, targeting 115 Gbp per genome)

DNA Inputs Outside the Validated Range

Low and high DNA inputs may be of interest to research scientists working with specific types of samples or applications. For instance, lower inputs (<100 ng) may be required when sample availability is limited or samples are irreplaceable. Such samples can include forensic, prenatal and pediatric samples, as well as formalin-fixed paraffin-embedded (FFPE) and fine needle aspiration (FNA) samples used in cancer research.

On the other hand, higher DNA inputs (>500 ng) may be desired to ensure greater library yields for sequencing, especially when planning to use certain sequencers such as Illumina's NovaSeq with SP and S1 flow cells. If you wish to use more than 1,000 ng of sheared DNA for library preparation, it is helpful to increase the volume or the concentration of the adapters used in the protocol. Contact Miroculus Technical Support for more information.

As a guide, the median library yields that can be obtained with Miro Canvas and manual preparation methods when using DNA inputs outside the validated range are shown in Table 2. Comparable library yields between Miro Canvas and manual preparation are obtained when using both low (<100 ng) and high (>500 ng) inputs of sheared human gDNA.

TABLE 2. MEDIAN LIBRARY YIELDS OBTAINED WITH LOW AND HIGH NA12878 DNA INPUTS WHEN USING DIFFERENT LIBRARY PREPARATION METHODS

Input (ng)	Median (range) library yields (nM)	
	Miro Canvas	Manually prepared
75	1.2 (0.3–1.9)	1.3 (1.0–1.6)
1,000	17.5 (13.1–23.4)	25.0 (18.3–32.4)

Summary

Miro Canvas is a DMF platform that facilitates library preparation for NGS. It is a versatile platform that boasts minimal hands-on time as well as automated protocols suitable for a variety of sequencers. Library yields obtained when using Miro Canvas are comparable to those obtained when using other common library preparation methods, both for DNA inputs inside and outside the validated range. Furthermore, WGS libraries prepared with Miro Canvas enable high-quality sequencing of the human genome.

References

1. Slatko BE et al. *Curr Protoc Mol Biol* 2018; 122 (1): e59.
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3. Chen Y-H et al. Poster presented at the American Society of Human Genetics (ASHG) 2019 Annual Meeting; Houston, USA, 15–19 October 2019.
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