

Automating the Oxford Nanopore Ligation Sequencing Kit on Miro Canvas[®]

Data from a demonstrated protocol

Key points

- Library preparation using the 1D ligation kit (LSK 109) from ONT is fully automated on Miro Canvas
- 25% reaction volumes compared to manual library preparation
- This protocol has been demonstrated on Miro Canvas using 1 µg high quality, high molecular weight input DNA
- 2.5 hr run time
- N50 comparable to manual

Introduction

Long read sequencing is particularly well suited for detection of large genomic mutations, coverage of long repeat regions that confound short read assemblies¹, and identification of signatures that can be lost due to PCR amplification (including relative abundance in metagenomic samples² and nucleotide modifications present on original DNA³).

ONT's long read sequencing of single-stranded DNA and RNA as they move through nanoscale pores has been a major technological achievement in genomic research^{3,4}. Its particular advantages within long read sequencing include being a small, portable sequencer that can be used in the laboratory and the field, requiring low capital cost, enabling rapid turnaround time, and having a user-friendly bioinformatics pipeline that allows for real-time analysis during sequencing².

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

Experimental workflow

The protocol was designed for fully automated use on Miro Canvas and has been tested using 1 µg of high quality, high molecular weight (HMW) input DNA. Before beginning, DNA should be quantified using a Broad-Range Qubit Quantification kit or similar. DNA repair and end prep, adapter ligation, and library clean-up are all automated on the Miro Canvas (Fig. 1). Downstream quantification requires hands-on time.



Figure 1. Experimental Workflow. The Miro Canvas automates all the steps following reaction setup, including: DNA repair and End prep, Post-Repair Bead Clean Up, Adapter Ligation, and Library Clean Up.



Results

For both manual library preparation and libraries prepared on Miro Canvas, 1 µg of Zymobiomics HMW DNA standard (D6322) was used as input. For both preparation types, volumes listed in the ONT Genomic DNA by Ligation Protocol (SQK-LSK109) were reduced to one-quarter. Each prepared library was loaded to a MinION flow cell (R9.4.1) and sequenced up to 4 hours. Libraries prepared using the automated workflow on Miro Canvas produced comparable read length distributions (Figure 2) and N50 read lengths (Table 1) to those prepared using the manual technique.

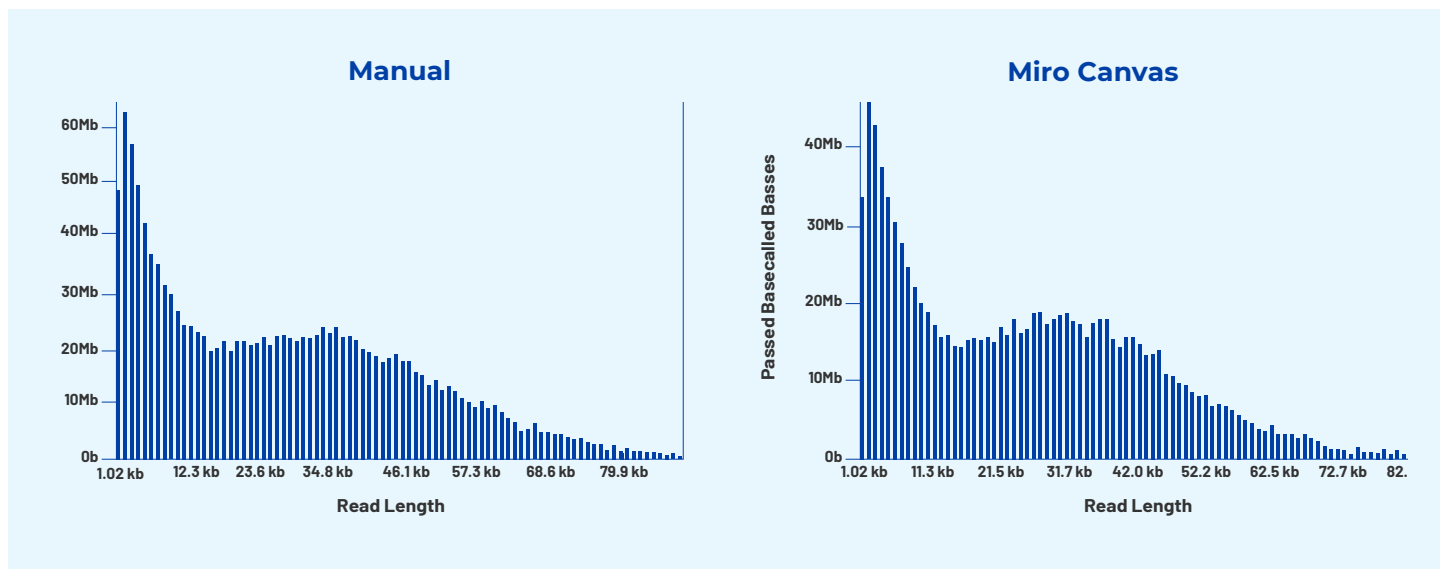


Figure 2. Read Length Distribution of Libraries Prepared Manually and Using Miro Canvas. Representative read length histogram Basecalled bases show a similar distribution for libraries prepared both manually and using Miro Canvas

SEQUENCING METRICS	MANUAL	MIRO CANVAS
Mean Read Length	6,051	5,981
Mean Read Quality	10.8	9.7
Median Read Length	1,663	1,756
Median Read Quality	11.4	10.1
Numbers of Reads	253,463	217,098
Read Length N50	23.71kb	23.06kb
Total Bases	1.556b	1.316b

Table 1. Sequencing Metrics Summary of Libraries Prepared Manually and Using Miro Canvas. Representative sequencing metrics for libraries prepared manually and using Miro Canvas are shown above. Read length and quality statistics are comparable between Miro Canvas and manual preparation.

The top five longest sequenced reads in Miro Canvas library were all longer than 120kb and of similar length to the top five longest ranked reads from the library prepared manually. Additionally, the longest read from the Miro Canvas library exceeded in length the longest read from the manually prepared library and had a higher mean call base quality score (Fig 3).

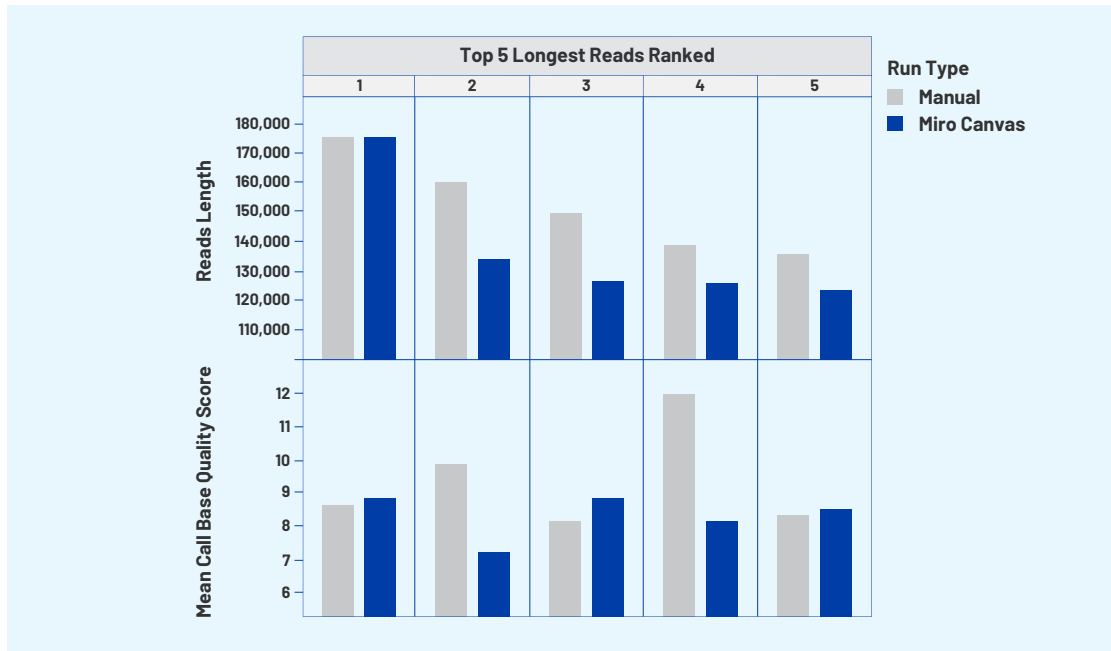


Figure 3. Read length and mean call base quality score of the top five longest reads sequenced

The Zymobiomics HMW DNA standard is composed of genomic DNA from seven bacteria and one yeast species. All of the eight expected species were identified through nanopore sequencing of libraries prepared both manually and using Miro Canvas. Figure 4 shows how the cumulative reads of each species compare between Manual and Miro Canvas runs.

Manual		Miro Canvas	
Salmonella	83,067	Salmonella	51,982
Escherichia	52,641	Escherichia	35,669
Enterococcus	20,011	Enterococcus	19,242
Staphylococcus	11,849	Staphylococcus	11,629
Pseudomonas	8,362	Pseudomonas	8,821
Listeria	8,015	Listeria	8,816
Bacillus	6,334	Bacillus	7,732
Shigella	2,134	Shigella	1,508
Saccharomyces	1,413	Saccharomyces	1,459

Figure 4. Mock Community Representation from Libraries Prepared both Manually and with Miro Canvas



Due to its compact nature, simple setup, and minimal infrastructure requirements (one, 120V adapter), the Miro Canvas is readily adaptable to support scientists outside of the laboratory and to facilitate collaboration between working groups. The Miro Canvas has been tested after air travel in carry-on baggage in a standard 20", hard shell suitcase with some additional padding (Fig 5).

Figure 5. Miro Canvas packed for air travel in carry-on baggage. The Miro Canvas's dimensions are 20.2cm (8in) W x 40.6cm (16in) D x 17.6cm (7in) H, making it an ideal travel companion to pair with the portable sequencers offered by Oxford Nanopore.



Summary

Miro Canvas is an advanced DMF platform that can be used to automate library preparation with the ONT Ligation Sequencing kit. When using the ONT Ligation Sequencing Protocol for Miro Canvas, the protocol is fully automated from DNA repair step to elution, and can be used with one-quarter reagent volumes. Both Miro Canvas and manual library preparation yield comparable results. Furthermore, its portability and compatibility with standard electrical sockets make it an ideal companion for the portable ONT sequencers for library preparation and sequencing beyond the walls of the traditional laboratory.

References

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Revisions

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