

Automating the Oxford Nanopore Ligation Sequencing Kit on MIRO CANVAS[®]

Introduction

Long read sequencing is particularly well suited for detection of large genomic mutations, coverage of long repeat regions that confound short read assemblies,¹ and identification of signatures that can be lost due to PCR amplification (including relative abundance in metagenomic samples² and nucleotide modifications present on original DNA).³

Oxford Nanopore Technologies (ONT) long read sequencing of single-stranded DNA and RNA moving through nanoscale pores has been a major technological achievement in genomic research.^{3,4} Its advantages include the use of a small, portable sequencer that can be deployed in the laboratory or the field, low capital cost requirements, rapid

turnaround times, and a user-friendly bioinformatics pipeline that allows real-time analysis during sequencing.²

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. This application note describes the results that can be expected when using the ONT Ligation Sequencing Kit in a protocol developed for the MIRO CANVAS. The resulting research use only libraries can then be sequenced using ONT sequencing platforms.

Key benefits:

- Library preparation using the 1D ONT Ligation Kit is fully automated on the MIRO CANVAS.
- 75 % reduction in reaction volumes compared to manual library preparation.
- This protocol has been demonstrated on MIRO CANVAS using 1 µg of high quality, high molecular weight input DNA.
- 2 hr 30 min run time.
- N50 comparable to manual library prep.

Overview: How to automate the ONT Ligation Sequencing Kit on MIRO CANVAS

MIRO CANVAS



Experimental set-up

The protocol was designed for fully automated use on the 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, post repair bead clean-up, adapter ligation and library clean-up are all automated on the MIRO CANVAS (**Figure 1**). Downstream quantification requires additional hands-on time.

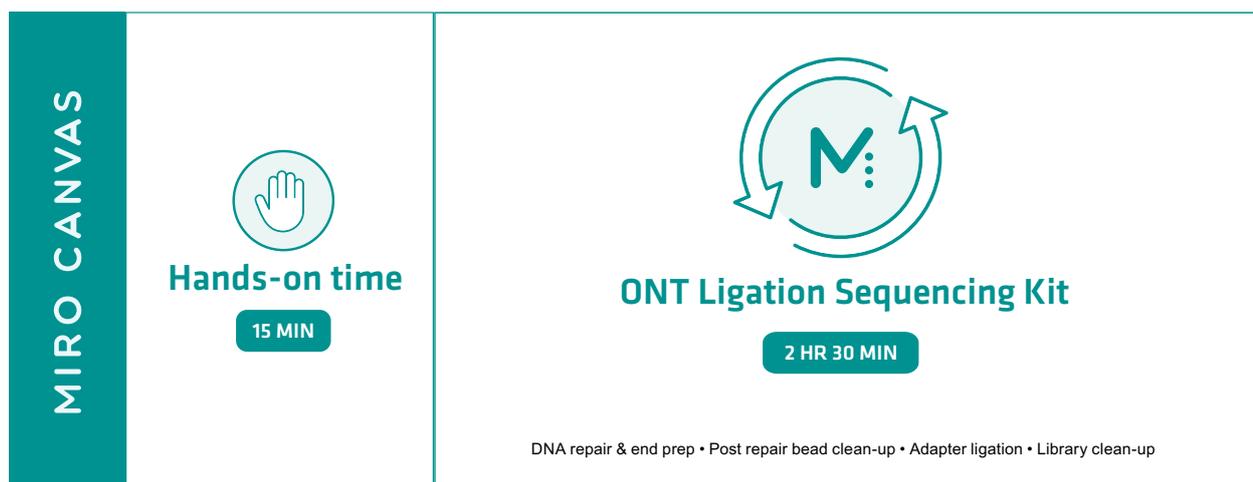


Figure 1: Experimental set-up. The MIRO CANVAS automates all the steps following reaction set-up, including DNA repair and end prep, post-repair bead clean-up, adapter ligation and library clean-up.

Results

For both manual library preparation and libraries prepared on the 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 by 75 %. Each prepared library was loaded into a MinION flow cell (R9.4.1) and sequenced for up to 3 hours. Libraries prepared using the automated workflow on the MIRO CANVAS produced comparable read length distributions (**Figure 2**) and N50 read lengths (**Table 1**) to those prepared using the manual technique.

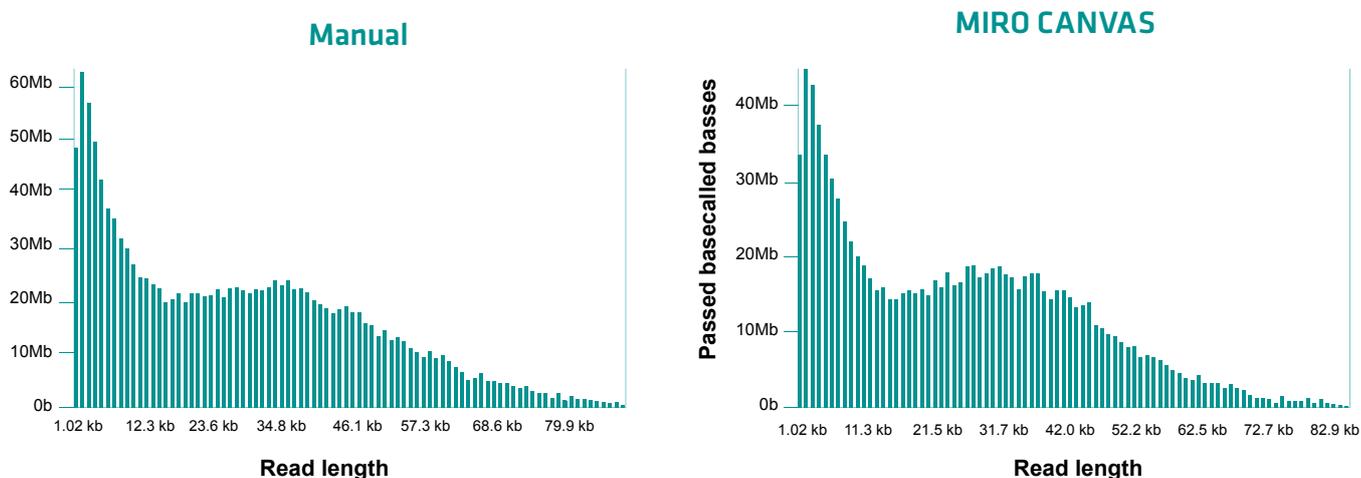


Figure 2: Read length distribution of libraries prepared manually and using the MIRO CANVAS. Representative read length histogram shows a similar distribution for libraries prepared both manually and using MIRO CANVAS.

Table 1: Summary of the sequencing metrics for libraries prepared manually and using the MIRO CANVAS. Representative sequencing metrics for libraries prepared manually and using the MIRO CANVAS are shown below. Read length and quality statistics are comparable between MIRO CANVAS and manual preparation.

SEQUENCING METRICS	MANUAL	MIRO CANVAS
Mean read length	6,051 kb	5,981 kb
Mean read quality	10.8 kb	9.7 kb
Median read length	1,663 kb	1,756 kb
Median read quality	11.4 kb	10.1 kb
Numbers of reads	253,463 kb	217,098 kb
Read length N50	23.71 kb	23.06 kb
Total bases	1.55 Gb	1.31 Gb

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

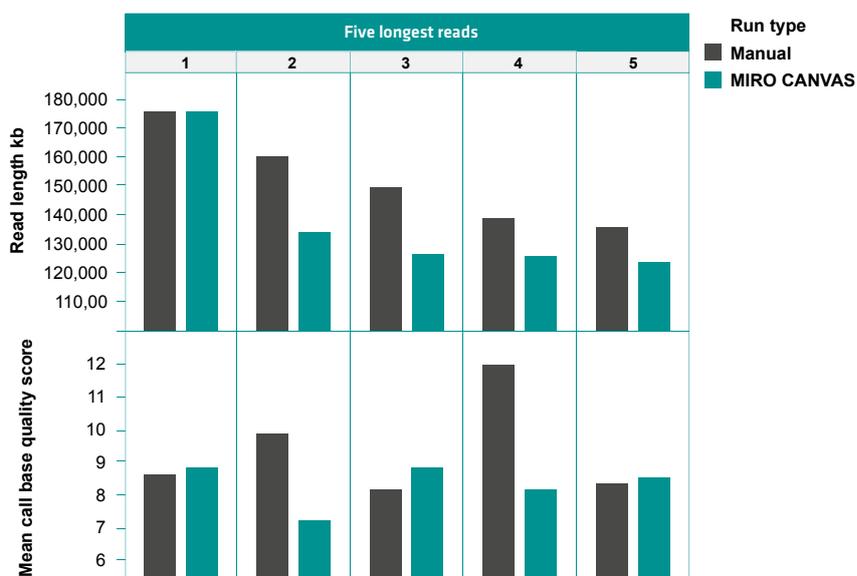


Figure 3: Read length and mean call base quality score for the five longest reads sequenced.

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 the MIRO CANVAS. **Figure 4** shows how the cumulative reads of each species compare between manual and MIRO CANVAS runs.

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

Figure 4: Cumulative read numbers for representative mock community using libraries prepared both manually and with the MIRO CANVAS.

Due to its compact nature, simple set-up and minimal infrastructure requirements (a 120V adapter), the MIRO CANVAS can support scientists outside of the laboratory and facilitate collaboration between working groups. The MIRO CANVAS has been tested after air travel in carry-on baggage and in a backpack (Figure 5).



Figure 5: The MIRO CANVAS's compact dimensions (20.2 cm W x 40.6 cm D x 17.6 cm H) make it easy to packed for travel, providing the ideal companion for ONT's portable sequencers.

Conclusion

- The MIRO CANVAS is an advanced DMF platform that can be used to automate library preparation with the ONT Ligation Sequencing Kit.
- The ONT Ligation Sequencing Protocol for the MIRO CANVAS is fully automated – from DNA repair step to elution – can reduce reagent volumes by 75 %, and yields results comparable to manual library preparation.
- Furthermore, its portability and compatibility with standard electrical sockets make it an ideal companion for highly portable ONT sequencers, offering library preparation and sequencing beyond the walls of the traditional laboratory.

References

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Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropploss	
Oxford Nanopore Technologies	SQK-LSK 109	Ligation Sequencing Kit	https://store.nanoporetech.com/ligation-sequencing-kit.html
Zymo Research	D6322	ZymoBIOMICS HMW DNA Standard	https://zymoresearch.eu/products/zymbiomics-hmw-dna-standard

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