Application Note

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Automating the Oxford Nanopore Ligation Sequencing Kit on the MIRO CANVAS

Introduction

Long-read sequencing is particularly well suited to the detection of large genomic mutations, coverage of long repeat regions that confound short-read assemblies,[1] and the identification of signatures that can be lost due to PCR amplification (including relative abundance in metagenomic samples[2] 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]

The MIRO CANVAS NGS prep system 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 V14 (LSK-114) 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 ONT Ligation Sequencing kit is fully automated on the MIRO CANVAS.
- 75 % reduction in reaction volumes compared to manual library preparation.
- This protocol has been demonstrated on the 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 the 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 Qubit™ dsDNA Quantification Assay Kit, Broad Range 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.

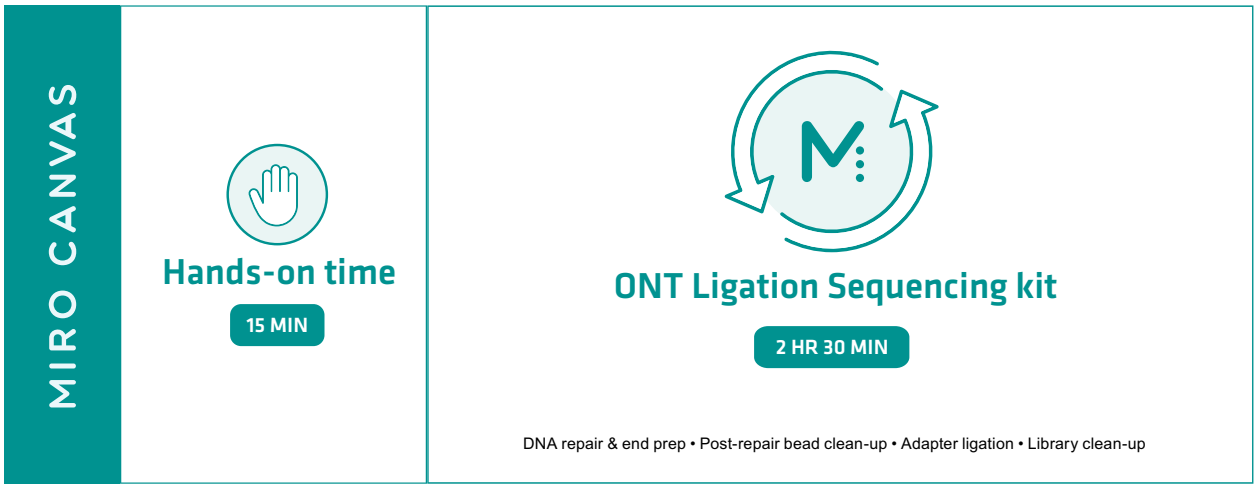

Figure 1: Experimental set-up. The MIRO CANVAS automates all the steps following reaction set-up, including DNA repair and end prep, post-repair bead clean-up, adapter ligation and library clean-up.

Results

1 µg of ZymoBIOMICS HMW DNA Standard was used as input for both manual library preparation and libraries prepared on the MIRO CANVAS, and the volumes listed in the ONT Ligation Sequencing Protocol were reduced by 75 %. Each library prepared was loaded into a MinION flow cell and sequenced for up to 3 hours. Libraries prepared using the automated workflow on the MIRO CANVAS produced read length distributions (**Figure 2**) and N50 read lengths (**Table 1**) comparable to those prepared using the manual technique.

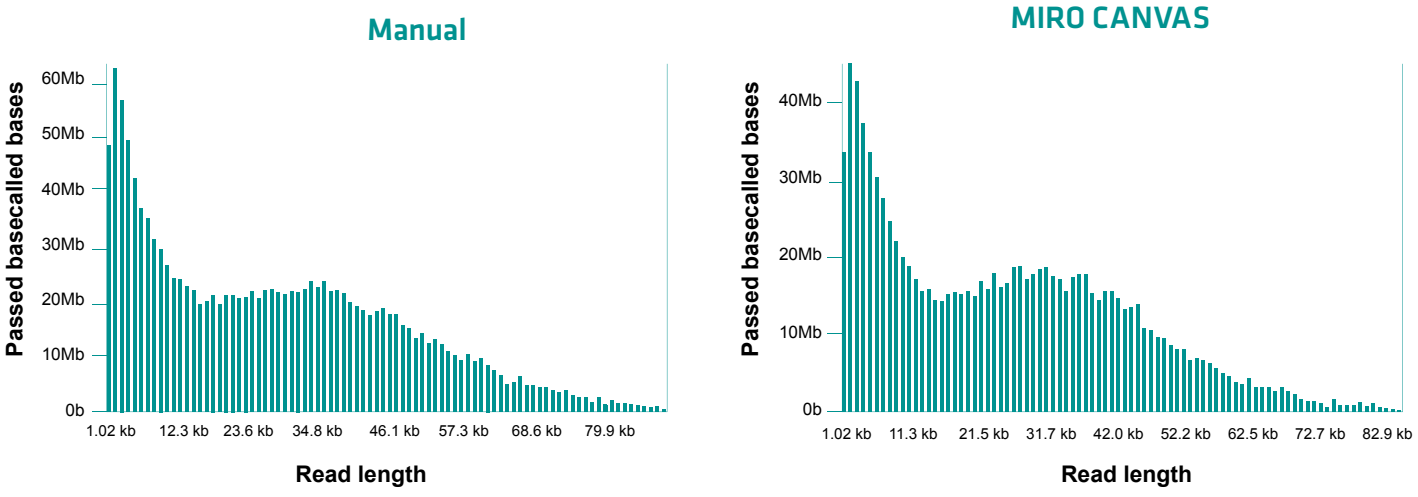

Figure 2: Read length distribution of libraries prepared manually and using the MIRO CANVAS. Representative read length histogram shows a similar distribution for libraries prepared both manually and using the MIRO CANVAS.

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

Table 1: Summary of the sequencing metrics for libraries prepared manually and using the MIRO CANVAS.

SEQUENCING METRICS	MANUAL	MIRO CANVAS
Mean read length	6051	5981
Mean read quality	10.8	9.7
Median read length	1663	1756
Median read quality	11.4	10.1
Numbers of reads	253,463	217,098
Read length N50	23.71 kb	23.06 kb
Total bases	1.55 Gb	1.31 Gb

The 5 longest sequenced reads in the MIRO CANVAS library were all longer than 120 kb, and of similar length to the 5 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**).

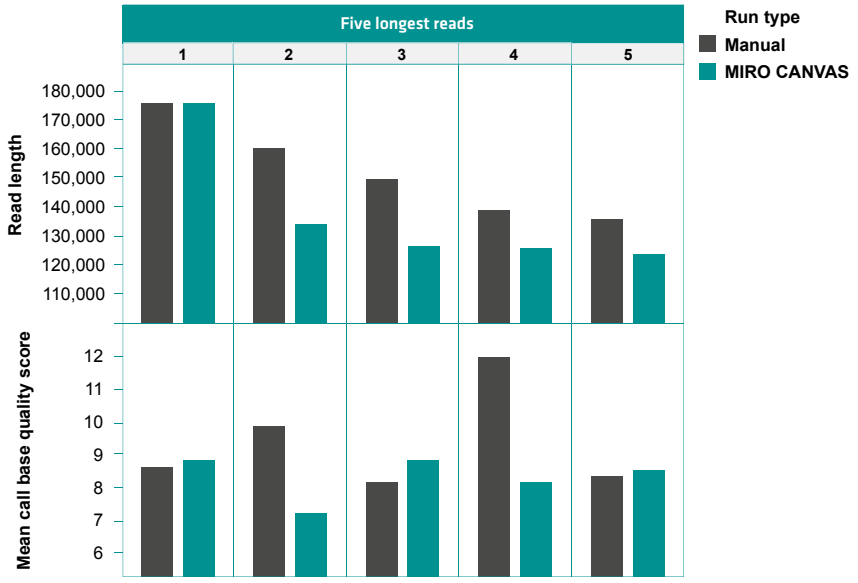

Figure 3: Read length and mean call base quality score for the 5 longest reads sequenced.

The ZymoBIOMICS HMW DNA Standard is composed of genomic DNA from 7 bacterial and 1 yeast species. All of the 8 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	
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	8362	*Pseudomonas*	8821
Listeria	8015	*Listeria*	8816
Bacillus	6334	*Bacillus*	7732
Shigella	2134	*Shigella*	1508
Saccharomyces	1413	*Saccharomyces*	1459

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

The compact nature, simple set-up and minimal infrastructure requirements (a 120 V adapter) of the MIRO CANVAS enable scientists to use it outside of the laboratory and aid 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 x 40.6 x 17.6 cm, w x d x h) make it easy to pack for travel, providing the ideal companion for ONT's portable sequencers.

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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 to elution – can reduce reagent volumes by 75 %, and yields results comparable to manual library preparation.
- The MIRO CANVAS's 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

1. Wick RR, Judd LM, Gorrie CL, Holt KE. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microbial genomics*. 2017 Sep 14;3(10):e000132. doi: 10.1099/mgen.0.000132. eCollection 2017 Oct. PMID: 29177090.
2. Petersen LM, Martin IW, Moschetti WE, Kershaw CM, Tsongalis GJ. Third-generation sequencing in the clinical laboratory: exploring the advantages and challenges of nanopore sequencing. *Journal of Clinical Microbiology*. 2020 Jan; 58(1): e01315-19. doi: 10.1128/JCM.01315-19. PMID: 31619531.
3. Deamer D, Akeson M, Branton D. Three decades of nanopore sequencing. *Nature Biotechnology*. 2016 May 6; 34(5): 518-524. doi: 10.1038/nbt.3423. PMID: 27153285.
4. Laver T, Harrison J, O'Niell PA, Moore K, Farbos A, Paszkiewicz K, Studholme DJ. Assessing the performance of the Oxford Nanopore Technologies MinION. *Biomolecular Detection and Quantification*. 2015 Mar;3:1-8. doi: 10.1016/j.bdq.2015.02.001. PMID: 26753127.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	M-01-0001-001-01	MIRO CANVAS NGS prep system	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-02-0001-001-03	MIRO Cartridge	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	M-03-0001-001-01	MIRO Dropgloss	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas

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