Application Note

INTEGRA

Automated library preparation for a nanopore DNA barcoding protocol on the MIRO CANVAS

Introduction

Long read sequencing allows the in-depth characterization of formerly unreadable regions, supporting de novo genome assembly and haplotype phasing.[1]

The Native Barcoding Kit (SQK-NBD114.24) uses DNA barcoding and multiplex nanopore sequencing to enable the identification of DNA molecules in a single sample that is being sequenced in parallel with other samples.[2] It can use PCR amplicons or non-amplified DNA.[3] This is important for applications where native DNA structure preservation is key – such as the analysis of base modifications, metagenomic and epigenetic studies – or when detecting species of interest, such as pathogens and their evasion mechanisms.[4]

The MIRO CANVAS NGS prep system is a digital microfluidics platform that automates NGS library preparation. It is compact (20.2x40.6x17.6 cm, WxDxH), requires minimal infrastructure (a standard 120V adapter), and has been shown to work after air travel in carry-on baggage and in a backpack.

This application note describes results from the Native Barcoding Kit partially automated on the MIRO CANVAS, which can handle up to 8 native barcoded samples or >60 bead purified amplicon samples.

Key benefits:

- Library preparation using the Native Barcoding Kit is partially automated on the MIRO CANVAS, which can process a barcoded pool of 8 end-prepped samples.
- This protocol uses ~3 µg of high quality, high molecular weight input DNA in the form of an 8-plex pool.
- The MIRO CANVAS enables a 50 % reduction in reaction volumes compared to manual library preparation.
- The automated protocol has a total run time of 2 hours and 40 minutes.
- N50 read lengths are comparable to manual library preparation.

Overview: How to automate the Native Barcoding Kit on the MIRO CANVAS

THE MIRO CANVAS

Experimental set-up

This experiment used 3.2 µg of short read eliminated (PacBio PN: SKU 102-208-300) ZymoBIOMICS HMW DNA Standard (Zymo research PN: D6322) as input for both manual library preparation and libraries prepared on the MIRO CANVAS. 400 ng of each ZymoBIOMICS HWM DNA Standard was quantified using a broad range Qubit™ quantification kit, followed by manual end-prep, unique barcode ligation and pooling to create an 8-plex pool. Reagent volumes listed in the Native Barcoding Kit protocol were reduced by 50 % after the barcoding step. The MIRO CANVAS protocol was then used to automate bead clean-up, adapter ligation and final library bead clean-up, which was also performed manually in parallel.

Figure 1: Experimental set-up for the Native Barcoding Kit workflow. Following manual pooling of end-prepped and barcoded DNA samples, the MIRO CANVAS automates pooled sample bead clean-up, adapter ligation and library clean-up.

Results

Each prepared library pool was loaded into a R10.4.1 MinION flow cell and sequenced until ~5 Gb of data was obtained. 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 manually.

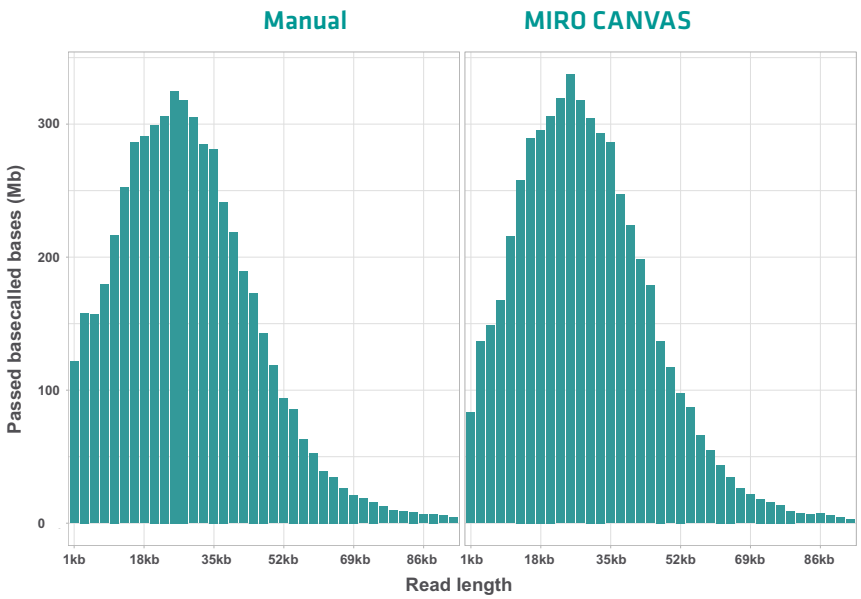

Figure 2: Representative read length histograms show similar distributions for libraries prepared manually or using the MIRO CANVAS.

Representative sequencing metrics for libraries prepared manually and using the MIRO CANVAS are shown below. Read length (bp) 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 (bp)	15,073	16,564
Mean read quality	10.5	9.9
Median read length (bp)	10,492	12,852
Median read quality	11.0	10.3
Numbers of reads	359,580	327,208
Read length N50 (bp)	27,592	27,843
Total bases	5.42 Gb	5.42 Gb

The 5 longest sequenced reads in the MIRO CANVAS library were all >150 kb, and greater in length than the 5 longest ranked reads from the manually prepared library pool (**Figure 3**). Additionally, the MIRO CANVAS had the higher mean call base quality score.

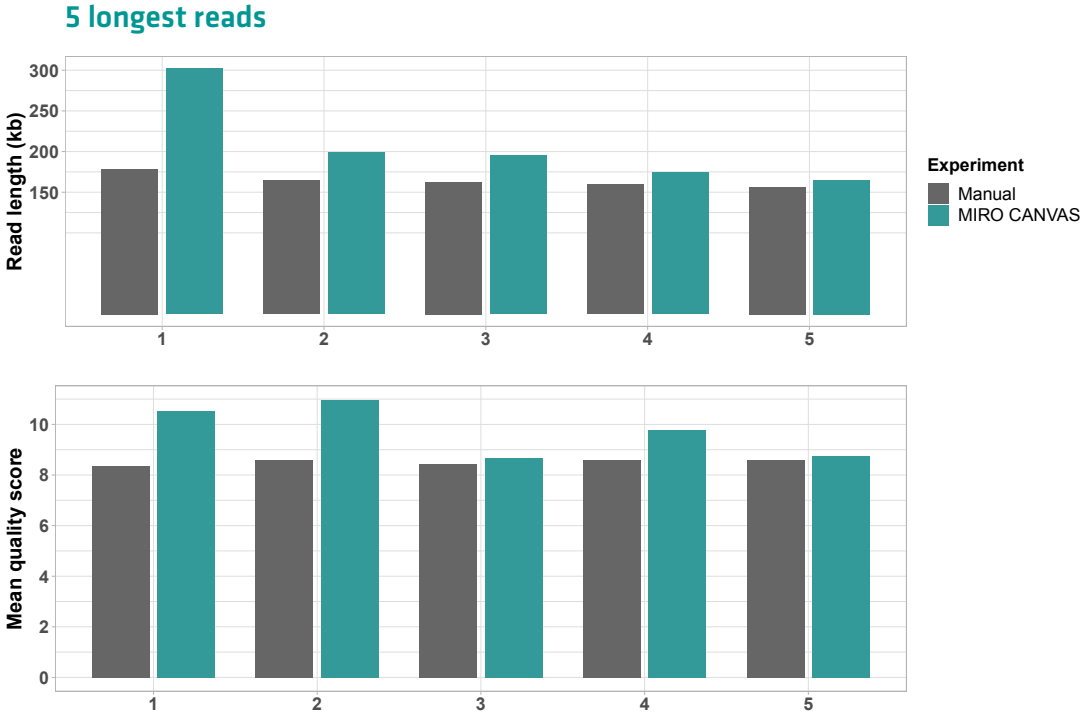

Figure 3: Read length and mean call base quality score for the five longest reads sequenced.

The ZymoBIOMICS HMW DNA Standard is composed of genomic DNA from 7 bacteria and 1 yeast species. All 8 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.

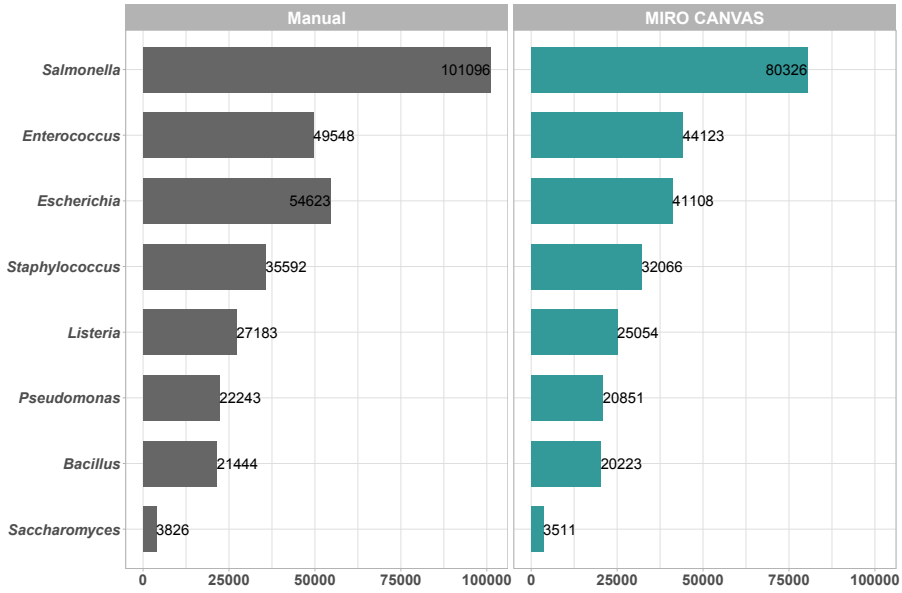

Figure 4: Cumulative read numbers for representative mock community per genus using libraries prepared manually and with the MIRO CANVAS.

The number of sequenced reads for each of the clinical samples was similar between pooled libraries for both the MIRO CANVAS and manual experiments. This reflected a balanced pool, where each barcode was +/-30 % read count on average, with no more than 20 % of barcodes falling outside the 30 % median bracket.

Similar results were obtained when the input DNA pool was comprised of pooled SARS-CoV-2 amplicons generated using the nanopore protocol pcr-tiling-SARS-CoV-2-nbd-PTCN_9103_v109_revR_13Jul2020-minion (data not shown, poster available upon request).

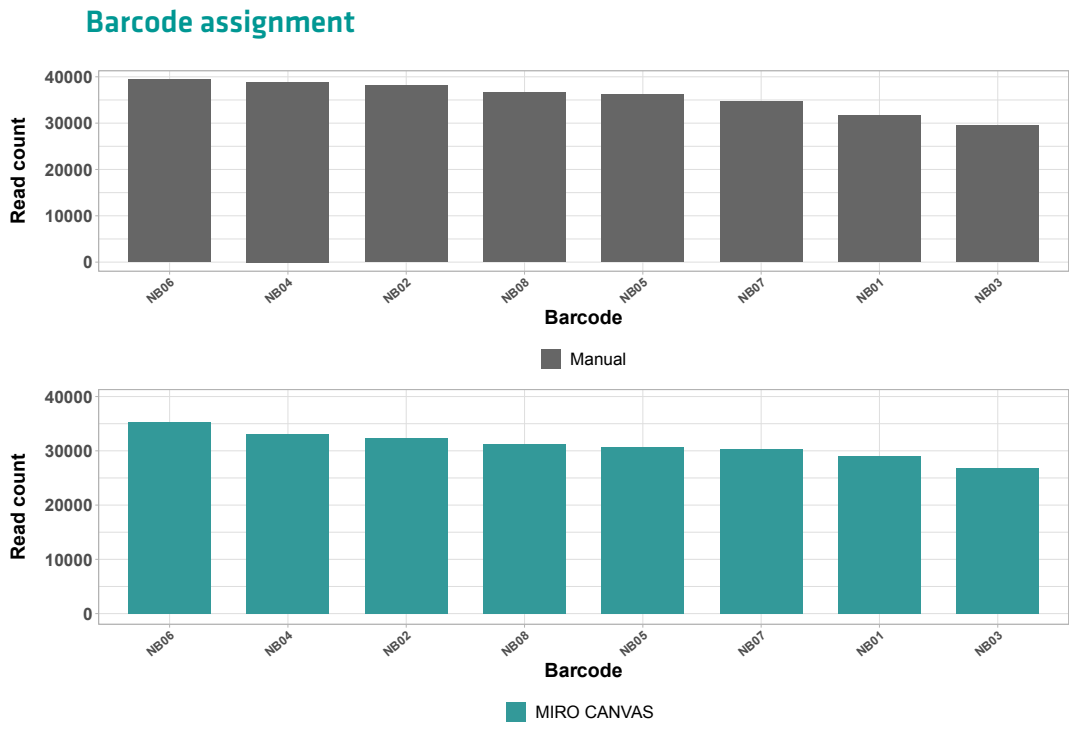

Figure 5: The number of sequenced reads for each one of the 8 uniquely barcoded samples in both the manually prepared and MIRO CANVAS processed pools.

Conclusion

- The MIRO CANVAS protocol uses the Native Barcoding Kit to automate the preparation of up to 8 samples for multiplex sequencing on a single flow cell.
- Following pooling, the entire process – from pooled sample bead clean-up to elution – is fully automated, reducing reagent requirements by 50 % and yielding results comparable to manual library preparation.
- For higher throughput sequencing, pools greater than 8-plex and/or amplicon DNA pools can be prepared on the MIRO CANVAS, as long as the pool volume is adjusted for loading compatibility.
- The system is portable, so can accompany highly portable ONT sequencers, offering multiplex library preparation and sequencing applicable to outbreak scenarios.

References

1. Bayliss SC, Hunt VL, Yokohama M, Thorpe HA, Feil EJ. The use of Oxford Nanopore native barcoding for complete genome assembly. GigaScience. 2017 Mar; 6(3): doi: 10.1093/gigascience/gix001. PMID: 28327913.
2. Florian T, Karen L, Emily F, Alon S, Hilary GM, Luis B, Bana J, Murat E. High molecular weight DNA extraction strategies for long-read sequencing of complex metagenomes. Microbiology Spectrum. 2022 Jul;22(5):1786-1802. doi: 10.1111/1755-0998.13588. PMID: 35068060.
3. Robert P, Kathleen V, Andrea S, Ellen F, Amanda E, Mihir SJ, Rebecca D, David R, Sarah G, Bruce G, Shanmuga S. Optimization of Oxford Nanopore Technology Sequencing Workflow for Detection of Amplicons in Real Time Using ONT-DART Tool. Genes (Basel). 2022 Oct 3;13(10):1785. doi: 10.3390/genes13101785. PMID: 36292670.
4. Mojnu M, Mohammad EH, Rashedul H, Md Shaheen A, Joynob AP, Md Mahmudul H, Ariful I, Sukanta C, Mohammed ZR. Culture-Independent Workflow for Nanopore MinION-Based Sequencing of Influenza A Virus. Genes (Basel). 2022 Oct. 3;13(10):1785. doi: 10.3390/genes13101785. PMID: 37212605

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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-02-0001-002-02	MIRO Cartridge, pack of 10 with MIRO Dropgloss (4 ml)	https://www.integra-biosciences.com/en/ngs-automation/miro-canvas
INTEGRA Biosciences	4012	Single Channel VIAFLO	https://shop.integra-biosciences.com/us/s/product/detail/01tD0000003aMXoIAM
INTEGRA Biosciences	3725	125 µl 10 ECO racks of 384 Tips	https://www.integra-biosciences.com/united-states/en/griptips/griptips-selector-guide
Oxford Nanopore Technologies	MIN-101B	MinION	https://store.nanoporetech.com/us/devices.html
Oxford Nanopore Technologies	SQK-NBD114-24	Native Barcoding Kit 24 V14	https://store.nanoporetech.com/us/native-barcoding-kit-24-v14.html
Oxford Nanopore Technologies	FLO-MIN114	Flow Cell (R10.4.1)	https://store.nanoporetech.com/flow-cell-r10-4-1.html
New England Biolabs	B6058S	NEBNext® Quick Ligation Reaction Buffer	https://www.neb.com/en-us/products/b6058-nebnext-quick-ligation-reaction-buffer
New England Biolabs	M0367L	Blunt/TA Ligase Master Mix	https://www.neb.com/en-us/products/m0367-blunt-ta-ligase-master-mix
ThermoFisher Scientific	15568025	UltraPure 1M Tris-HCl, pH 8.0	https://www.thermofisher.com/order/catalog/product/15568025
ThermoFisher Scientific	AM9760G	NaCl (5 M), RNase-free	https://www.thermofisher.com/order/catalog/product/AM9760G?SID=srch-hj-AM9760G
ThermoFisher Scientific	Q32850	Qubit™ dsDNA BR Assay Kit	https://www.thermofisher.com/order/catalog/product/Q32850?SID=srch-srp-Q32850
ThermoFisher Scientific	Q33238	Qubit 4 Fluorometer	https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238
Sigma-Aldrich	E7023	Ethyl alcohol, Pure	https://www.sigmaaldrich.com/US/en/product/sigald/e7023
VWR	NUPW-0050	Nuclease-free water	https://us.vwr.com/store/product/15299612/water-for-molecular-biology-nuclease-free
Eppendorf	022431021	DNA LoBind® Tubes	https://www.eppendorf.com/us-en/Products/Laboratory-Consumables/Tubes/DNA-LoBind-Tubes-p-022431021
USA Scientific	1402-4700	TempAssure PCR Flex-Free 8-Tube Strips, natural color 0.2mL	https://www.usascientific.com/flex-free-pcr-8-strip-attached-clear-flat-caps/p/PCR-Tu-Flex-Att-Optic
Scientific Industries	SI-0236	Vortex-Genie 2	https://www.scientificindustries.com/vortex-genie-2.html

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