Application Note

INTEGRA

The MIRO CANVAS NGS prep system enables fully automated library prep for nanopore human whole genome sequencing (WGS)

Introduction

Long-read sequencing has advanced genomics by resolving complex genomic regions and detecting structural variants often missed by short-read technologies.[1] Nanopore-based WGS is actively being validated for clinical use,[2] demonstrating its ability to rapidly solve complex cases[3] and potential for routine genetic testing.[4] The Oxford Nanopore Technologies (ONT) Ligation Sequencing Kit V14 (SQK-LSK114) enables library prep from high input and high molecular weight (HMW) DNA, achieving 30x human genome coverage and an N50 of 30 kb when sequenced using a PromethION device. The MIRO CANVAS simplifies library prep, reducing hands-on time and minimizing variability. This application note presents results from fully automated library prep of human whole genomes using 30 kb and 10 kb samples.

Key benefits:

- Library preparation for human WGS using the Ligation Sequencing DNA V14 kit is fully automated on the MIRO CANVAS platform.
- This MIRO CANVAS protocol supports higher inputs of up to 5 µg of HMW DNA, even using >30 kb samples.
- The MIRO CANVAS enables a 75 % reduction in reaction volumes compared to manual library preparation.
- The system provides 2 hours and 50 minutes of walk-away time for automated library preparation.
- The MIRO CANVAS protocol delivers sequencing yields and metrics comparable to those obtained through manual preparation, supporting reliable downstream analysis.

Overview: How to automate ligation sequencing DNA V14 for high input on the MIRO CANVAS

MIRO CANVAS NGS PREP SYSTEM

Experimental set-up

In this experiment, 3 µg of short-read eliminated GM24385/HG002 HMW DNA sheared down to 10 kb and 30 kb was used as inputs for both manual library preparation and the fully automated workflow on the MIRO CANVAS.

HMW DNA was extracted from the GM24385 cell line (Coriell) using the Monarch® HMW DNA Extraction Kit for Cells & Blood (New England Biolabs, #T3050). The PacBio Short Read Eliminator (SRE) Kit (PacBio, #102-208-300) was used to remove DNA fragments smaller than 10 kb. 10 µg of the purified and SRE-treated HMW DNA was mechanically sheared using a g-TUBE (Covaris, #520079) at 5,600 rpm to target a peak size of 10 kb. Another 10 µg of DNA was sheared using the Megaruptor® 3 (Hologic Diagenode, #B06010003) with a Megaruptor 3 Shearing Kit (Hologic Diagenode, #E06010003, #E07010003) at speed 25 and a concentration of 25 ng/µl to target a peak near 30 kb. Both sheared DNA samples were analyzed using the Femto Pulse System (Agilent Technologies, Inc., #M5330AA) with the Genomic DNA 165 kb Kit (Agilent Technologies, Inc., #FP-1002-0275), as shown in **Figure 1**.

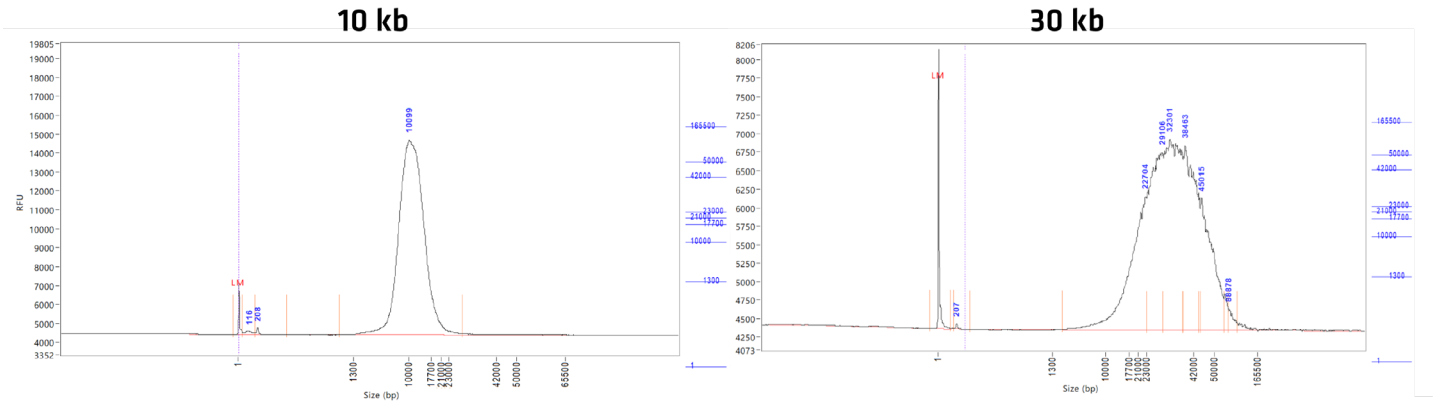

Figure 1: Femto Pulse traces of GM24385/HG002 HMW DNA post shearing. Left: 10kb Covaris sheared sample peaking at 10,090 bp. Right: 30kb Megaruptor sheared sample peaking at 32,301 bp.

Manual libraries were prepared using full-scale standard reaction volumes of the ONT Ligation Sequencing Kit V14. The MIRO CANVAS protocol was then used to automate DNA repair and end-prep, adapter ligation, and library clean-up steps at quarter-scale reaction volume (saving 75 % on reagents). The total run time for this automated process was 2 hours and 50 minutes (**Figure 2**).

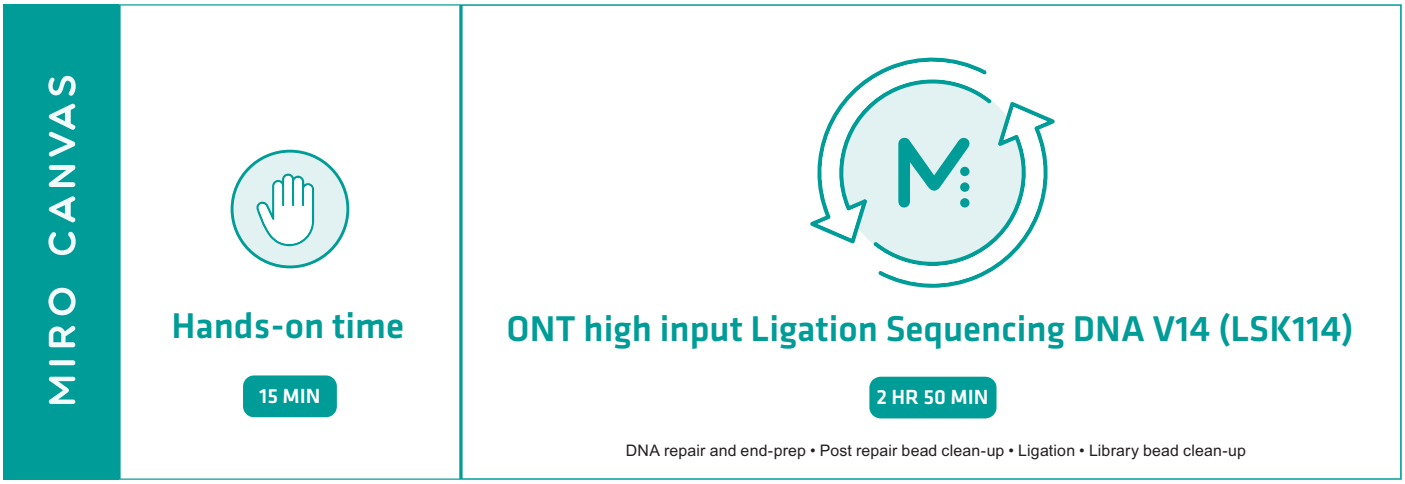

Figure 2: Experimental set-up for the high input ONT Ligation Sequencing DNA V14 workflow. Input DNA and reagents are loaded into the MIRO Cartridge and the MIRO CANVAS system automates the steps of DNA repair and end-prep, post-repair bead clean-up, adapter ligation and library clean-up.

Product library mass was measured using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific, #Q33226) with the Qubit dsDNA Broad Range (BR) Assay Kit (Thermo Fisher Scientific, #Q32850). Libraries were sequenced on a PromethION 2 Solo device (Oxford Nanopore Technologies, #PRO-SEQ002). A total of 250 ng of the 10 kb libraries, and 450 ng of the 30 kb libraries, were loaded onto PromethION Flow Cells (Oxford Nanopore Technologies, #FLOPRO114M). Data collection for the 10 kb sample was performed using a single loading of a PromethION Flow Cell, reaching >30x coverage by the flow cell's end of life. To maximize the sequencing yield for the 30 kb libraries, the flow cells were washed and reloaded twice – a total of 3 loading cycles – and data was collected over 72 h to reach ~105 Gb. High accuracy base calling was performed for all 4 libraries, and modified bases were also called for 10 kb libraries.

After mapping the reads, unmapped reads were excluded, and key sequencing metrics – F1 scores by variant types, coverage of difficult loci and methylation profiles – were compared between 10 kb and 30 kb libraries prepared manually or using the MIRO CANVAS.

Results

The MIRO CANVAS yielded product libraries within the same range as manual preparations for both 10 kb and 30 kb samples (**Table 1**), allowing sufficient material for PromethION Flow Cell loading with 20-35 fmol of library in each sequencing run. The MIRO CANVAS recovered 42 µl for each library, as shown in **Table 1**, which can be further diluted to load the flow cell again after washing, for extended sequencing data collection.

Table 1: Recovered volumes and concentrations for 10 kb and 30 kb library samples prepared manually and using the MIRO CANVAS.

LIBRARY PREP METHOD	INPUT DNA LENGTH (KB)	RECOVERY VOLUME (µL)	LIBRARY CONCENTRATION (NG/µL)	TOTAL LIBRARY MASS (NG)
Manual	10	33	50.2	1,656 (55 %)
	30	96	16.5	1,584 (53 %)
MIRO CANVAS	10	42	28.1	1,180 (40 %)
	30	42	35.6	1,495 (50 %)

Pore occupancy remained over 90 % throughout the sequencing runs, and the pore activity status showed near-zero adapter presence in the MIRO CANVAS libraries, implying efficient clean-up (data not shown). High pore occupancy with low adapter presence resulted in a high sequencing yield, accomplishing >30x coverage of the human genome for both the manual and MIRO CANVAS libraries.

Libraries prepared using the automated workflow on the MIRO CANVAS produced read length distributions (**Figure 3**) and N50 reads (**Table 2**) comparable to the manual process. For the 30 kb sample, the MIRO CANVAS sequencing results show a higher representation of read lengths >30 kb, leading to a higher N50 score compared to that of a manually prepared library (**Table 2**).

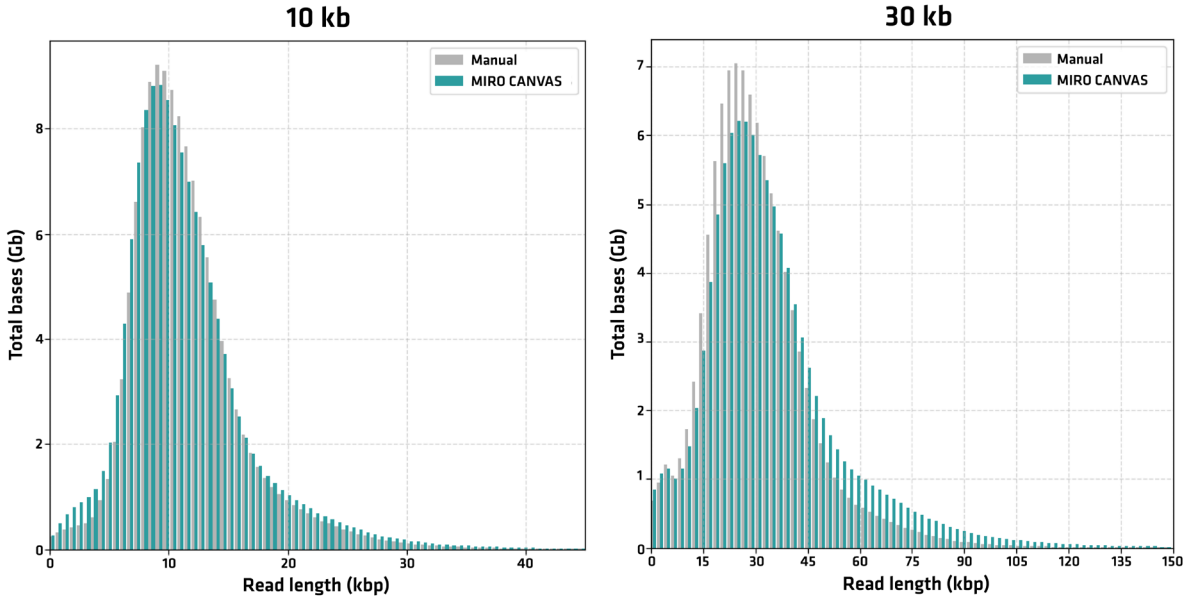

Figure 3: Overlaid graphs showing total bases by read length for manual libraries (gray) and MIRO CANVAS libraries (teal). Left: 10 kb manual and MIRO CANVAS libraries. Right: 30 kb manual and MIRO CANVAS libraries.

Table 2 summarizes key sequencing metrics across preparation methods and sample types. The MIRO CANVAS results were comparable to manually prepared libraries for the mean and median read lengths, the median read quality of >Q20, and human genome coverage for both 10 kb and 30 kb, with near-matching numbers of reads and dataset sizes in terms of sequenced bases. The N50 score in the 30 kb sample was higher for the MIRO CANVAS, due to the increased representation of longer reads, as shown in **Figure 3**.

Table 2: Summary of key sequencing metrics for libraries prepared manually and using the MIRO CANVAS.

INPUT DNA	10 KB		30 KB	
PREPARATION METHOD	MANUAL	MIRO CANVAS	MANUAL	MIRO CANVAS
Number of reads (M)	14.5	16.0	5.5	5.4
Base (Gb)	134.1	137.9	104.1	105.6
Mean read length (bp)	9,242	8,645	19,076	19,668
Median read length (bp)	9,149	8,484	18,797	18,462
N50 (bp)	10,925	10,625	28,916	31,253
Median read quality	20.31	20.11	20.37	20.38
Total coverage (x)	43.4	44.7	33.8	34.3

To assess the performance of libraries prepared using the MIRO CANVAS for applications requiring human genome variant detection, the system was used to detect single nucleotide variants (SNVs), insertions-deletion (INDELs), structural variants (SVs), and SVs of challenging medically-relevant genes (SV-CMRGs), and the respective F1 scores were computed (**Table 3**). Concordant to the comparable key sequencing metrics, MIRO CANVAS libraries presented equal or better F1 scores for SNVs, INDELs, SVs and SV-CMRGs compared to manually prepared libraries.

Table 3: Comparison of manual and MIRO CANVAS F1 scores across variant types.

	10 KB		30 KB	
VARIANT TYPE F1 SCORE	MANUAL	MIRO CANVAS	MANUAL	MIRO CANVAS
SNV	99.8 %	99.8 %	99.7 %	99.7 %
INDEL	87.0 %	86.9 %	85.7 %	85.5 %
SV	94.5 %	95.0 %	95.4 %	95.5 %
SV-CMRG	93.5 %	93.3 %	92.9 %	92.9 %

SV-CMRGs are clinically relevant, and are known to be difficult to resolve with short-read sequencing due to their complexity and repetitiveness. In the nanopore sequencing datasets analyzed for this study, the F1 score of >92 % for all SVs confirmed that the MIRO CANVAS libraries represented these difficult loci well. Read mapping across the STRC gene – a CMRG located at 15q15.3 – was visualized in the Integrated Genome Viewer (IGV) to compare coverages between the manual and MIRO CANVAS libraries for both 10 kb and 30 kb samples (**Figure 4**). Modified reads were also visualized from the 10 kb libraries across the Paternally Expressed Gene 3 (PEG3) locus at 19q13.4, revealing patterns of imprinting where maternal chromosome methylation impairs gene expression. In both of these examples, the MIRO CANVAS delivered coverage equal to, or better than, manually prepared libraries. Visualization in the IGV revealed coverage of the CFC1B gene – which is known to be challenging to detect – and found to be covered only in the 30 kb sample prepared using the MIRO CANVAS (data not shown).

Figure 4: Coverage comparison across the STRC gene for both 10 kb and 30 kb samples (left) and coverage plus methylation profile comparison across the PEG3 gene for 10 kb samples (right).

Conclusion

- The MIRO CANVAS protocol has been optimized for high input and HMW DNA. Fully automated library preparation with minimal hands-on time is achieved using the Ligation Sequencing Kit V14, enabling a complete walk-away experience.
- Automation reduces reagent consumption by 75 % compared to manual preparation, while delivering sequencing results that are comparable in pore occupancy (>90 %) and quality (>Q20).
- Both 10 kb and 30 kb human genome libraries achieved over 30x coverage from a single PromethION Flow Cell, with quality sequencing metrics validating the system's reliability.
- MIRO CANVAS library preparation for long-read sequencing technologies enhances the detection of clinically relevant SVs and enables epigenetic studies through methylation profiling.

References

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3. Goenka SD et al. Accelerated identification of disease-causing variants with ultra-rapid nanopore genome sequencing. Nat Biotechnol. 2022 Mar 28;40(7):1035-1041. http://doi.org/10.1038/s41587-022-01221-5
4. Kaplun A, Krautz-Peterson G, Neerman N, Stanley C, Hussey S, Folwick M, McGarry A, Weiss S, Kaplun A. ONT long-read WGS for variant discovery and orthogonal confirmation of short read WGS derived genetic variants in clinical genetic testing. Front. Genet. 2023 April. https://doi.org/10.3389/fgene.2023.1145285

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	VIAFLO single channel 125 µl electronic pipette	https://www.integra-biosciences.com/united-states/en/electronic-pipettes/viaflo
INTEGRA Biosciences	6565	125 µl Sterile, Filter Low retention GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
Oxford Nanopore Technologies	PRO-SEQ002	PromethION 2 Solo	https://nanoporetech.com/products/sequence/promethion-2
Oxford Nanopore Technologies	SQK-LSK114	Ligation Sequencing Kit V14	https://nanoporetech.com/document/genomic-dna-by-ligation-sqk-lsk114
Oxford Nanopore Technologies	FLO-PRO114M	PromethION Flow Cell Packs (R10.4.1)	https://nanoporetech.com/products/sequence/promethion
New England Biolabs	T3050	Monarch HMW DNA Extraction Kit for Cells & Blood	https://www.neb.com/en-us/products/t3050-monarch-hmw-dna-extraction-kit-for-cells-and-blood
PacBio	102-208-300	SRE Kit	https://www.pacb.com/products-and-services/consumables/sample-prep-kits/
Covaris	520079	g-TUBE	https://www.covaris.com/g-tube-pr520079
Hologic Diagenode	B06010003	Megaruptor 3	https://www.diagenode.com/en/p/megaruptor-3
Hologic Diagenode	E07010003	Megaruptor 3 Shearing Kit	https://www.diagenode.com/en/p/megaruptor-3-shearing-kit
Agilent Technologies, Inc.	M53330AA	Femto Pulse System	https://www.agilent.com/en/product/automated-electrophoresis/femto-pulse-systems/femto-pulse-system/femto-pulse-system-365750
Agilent Technologies, Inc.	FP-1002-0275	Genomic DNA 165 kb Kit	https://www.agilent.com/en/product/automated-electrophoresis/femto-pulse-automated-pulsed-field-capillary-electrophoresis/femto-pulse-system-dna-analysis-kits/genomic-dna-165-kb-kit-365739
Thermo Fisher Scientific	Q33226	Qubit 4 Fluorometer	https://www.thermofisher.com/order/catalog/product/Q33226
Thermo Fisher Scientific	Q32850	Qubit dsDNA BR Assay Kit	https://www.thermofisher.com/order/catalog/product/Q32850

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