Miroculus Miro Canvas Device Use with Nanopore Sequencing Protocols

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Abstract

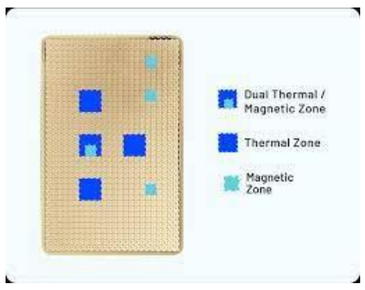
DEVCOM CBC is developing multiple workflows with MinION nanopore sequencing systems, from Oxford Nanopore Technologies (ONT), for rapid sample identification by a wide range of end-users (i.e., those with minimal laboratory training to those proficient in molecular biology techniques). Sequencing efforts currently provide a large amount of data in a relatively short amount of time; however, decreasing the amount of hands-on time is key in making the technology more practical and accessible, even for proficient users. Miroculus has provided an alternative to this time-consuming, hands-on process with their Miro Canvas device. Miroculus has previously demonstrated success with ONT's genomic DNA utilizing ligation protocols, SQK-LSK 109, 110, 112, and 114. To assess the utility of this device in the hands of laboratory trained personnel, we tested its ability to successfully produce libraries ready for sequencing with the ONT rapid sequencing kit, SQK-LSK110, with plans to test additional versions in the future. The Miro Canvas device required upfront loading of reagents, including a DNA sample, which took approximately 5-10 minutes. For this experiment, we used DNA from Lambda bacteriophage. Once loaded, the instrument completed library preparation in roughly 2 hours and 40 minutes. The Lambda DNA sample was retrieved and prepared for sequencing on a MK1C device adapted with software for species identification in real time and we observed that Lambda was correctly assigned by the software in less than 10 minutes. The flow cell was then transferred to a GridION and, within 20 hours, 2.08 million base calls were acquired. Over 1.9 million of the base calls passed quality control (QC) measures. However, DEVCOM CBC is pursuing the development of a faster process that will also extract and purify DNA from a lysed biological sample prior to performing library preparation. Once a lysed sample has been added to the Miro Canvas device, DNA is purified using an AmpureXP bead-based approach, followed by library preparation using ONT's Rapid Sequencing Kit. The amount of Dropgloss that is used to maneuver small reagent volumes within the system was minimized so that it would not interfere with sequencing. DEVCOM CBC continues to work towards automation of the full process, from sample and reagent loading to sample retrieval, but the samples generated using the current process demonstrated promising results using a lysed Escherichia coli sample. Species identification in real time was observed in less than 10 minutes using our modified Mk1C device. After transferring the flow cell onto the GridION for 20 hours, 2.25 million base reads were generated with over 2 million of those passing QC. Once a fully automated process has been implemented, we will further streamline the process to reduce time. Successful implementation will offer personnel with some laboratory training an effective alternative to hands on library preparation processes, allowing for time devoted to other laboratory endeavors.

Introduction

- Decreasing hands on time for library preparation can make sequencing more practical and accessible to the laboratorian
- Previous success has been seen with numerous liquid handling devices
- Miroculus announced their commercial Miro Canvas device in 2022, with initial workflow for Illumina library preparation as well as Oxford Nanopore Technologies ligation sequencing kit protocols
- Successful implementation of ONT's rapid kits with the Miro Canvas device would lead to a large reduction in time preparing a sample for a sequencing run, with the added benefit of minimal hands on time for the user

Methods

- The Miro Canvas device utilizes specific cartridges that allow for proper movement across the device to make use of the on device magnets and heaters
- In order to effectively move small or particularly viscous reagents proprietary drop gloss is added before addition to the cartridge
- Preparation of reagents with drop gloss and addition of sample and reagents takes around 15 minutes



- We first tested the workflow for ONT's ligation sequencing SQK-LSK110 kit and Lambda DNA with the Miro Canvas device, following the previously tested protocol from Miroculus
- The 2nd test performed utilized ONT's rapid sequencing SQK-RAD004 kit and *Escherichia coli*, following a new protocol developed through a mutual effort between Miroculus and CCDC CBC employees. Purification using a magnetic-bead based approach and library preparation are performed by the device. We are still working through full automation of the process.

Results

- Initial testing with SQK-LSK110 and Lambda, organized results for output from Centrifuge classification tool

Barcode Sample	Total Reads	Organism	Reads	Unique Reads	% Unique Reads	Conc (ng/uL)
No Barcode Miro Canvas Trainiing SQK LSK-110 Ligation Kit	1,910,677	Escherichia coli	886,535	886,535	100.0	NA
		Escherichia virus Lambda	304,041	304,041	100.0	
		Escherichia coli B	28,840	28,840	100.0	
		Escherichia coli 'BL21-Gold(DE3)pLysS AG'	22,008	22,008	100.0	
		Escherichia coli K-12	7,785	7,785	100.0	
		Escherichia coli O16:H48	5,326	5,326	100.0	
		Escherichia coli str. K-12 substr. MG1655	1,989	1,989	100.0	
		Enterobacteriaceae	1,690	1,690	100.0	
		Escherichia			#DIV/0!	

Results Continued

- Initial testing with SQK-LSK110 and Lambda, organized results for output from MiniMap2 alignment tool utilizing NCBI's RefSeq database

Organism	Genome Size	Reads Mapped	Unique Reads	% Unique Reads
[strain|Escherichia coli K-12; strain K-12 C3026] chromosome, complete genome	4,745,335	2,323,157	20	0.0
[strain|Escherichia coli B; strain C3029] complete genome	4,569,265	1,481,868	3	0.0
[species|Lambdavirus lambda] complete genome	48,582	911,385	45	0.0
[species|Escherichia coli; strain BH212] chromosome, complete genome	4,697,261	322,666	298	0.1
[no rank|Escherichia coli O16:H48; strain PG20180173] chromosome, complete genome	4,656,833	220,550	2,771	1.3
[no rank|Escherichia coli O16:H48; strain PG20180050] chromosome, complete genome	4,615,393	220,438	2,778	1.3
[species|Escherichia coli;strain J53] chromosome, complete genome	4,682,654	220,199	233	0.1
[no rank|Escherichia coli O16:H48; strain PG20180060] chromosome, complete genome	4,668,151	220,190	2,795	1.3
[no rank|Escherichia coli O16:H48; strain PG20180052] chromosome, complete genome	4,615,431	220,099	2,754	1.3

- Testing with SQK-RAD004 and E. coli, organized results for output from Centrifuge classification tool

Barcode Sample	Total Reads	Organism	Reads	Unique Reads	% Mapped of Total Reads	Conc (ng/uL)
No Barcode Miro Canvas Training SQK-RAD004	2080380	Escherichia coli	1020799	1020799	49.067911	NA
		Enterobacteriaceae	287223	287223	13.806276	
		root	210005	210005	10.09455	
		Escherichia coli BW2952	56961	56961	2.7380094	
		Shigella boydii	53736	53736	2.5829896	
		Shigella dysenteriae	50364	50364	2.4209039	
		Escherichia coli KO11FL	48263	48263	2.3199127	
		Shigella flexneri	27237	27237	1.309232	
		Escherichia	18489	18489	0.8887319	
		Shigella	17194	17194	0.8264836	

- Testing with SQK-RAD004 and E. coli, organized results for output from MiniMap2 alignment took utilizing the RefSeq database

Organism	Genome Size	Reads Mapped	Unique Reads	% Unique Reads
[species|Escherichia coli] Escherichia coli strain ER1709 chromosome, complete genome	4,582,922	86,977	10,995	12.6
[strain|Escherichia coli KLY] Escherichia coli KLY chromosome, complete genome	4,718,625	86,784	2	0.0
[-|Unrecognized organism] Escherichia coli strain NCTC12655 chromosome 1	4,757,404	82,120	3	0.0
[strain|Escherichia coli K-12] Escherichia coli K-12 strain K-12 MG1655 chromosome, complete genome	4,682,166	79,775	174	0.2
[species|Escherichia coli] Escherichia coli strain tolC- chromosome, complete genome	4,792,280	77,535	7,265	9.4
[species|Escherichia coli] Escherichia coli strain BE104 chromosome, complete genome	4,775,202	75,713	7,695	10.2
[strain|Escherichia coli K-12] Escherichia coli K-12 strain K-12 MG1655 chromosome, complete genome	4,660,512	72,730	380	0.5
[strain|Escherichia coli K-12] Escherichia coli K-12 strain K-12 DHB4 chromosome, complete genome	4,546,929	68,910	41	0.1
[strain|Escherichia coli K-12] Escherichia coli K-12 strain K-12 C3026 chromosome, complete genome	4,745,335	65,781	42	0.1

Conclusion

- Successful use of the Miro Canvas device allows a 2 hour hands-on protocol to be reduced to only about 15 minutes of hands on time
- There are a few difficulties to be further investigated primarily with the rapid sequencing kit. Initial tests necessitated override to acquire sample, and varying amounts of drop gloss were tested to ensure proper distribution of RAP reagent

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