DNA Loading for Oxford Nanopore MinION

Sequencing Guide v1 (01/2020)

Kits Required

The major kits used in this application note are listed below. Additional equipment and materials required by each kit are provided in the kit specific protocols.

Kit	Model
Nanobind CBB Big DNA Kit	Circulomics (NB-900-001-01)
Ligation Sequencing Kit	Oxford Nanopore Technologies (SQK-LSK109)
MinION / GridION Flow Cell	Oxford Nanopore Technologies (FLO-MIN106D)

DNA Loading Guidelines

This Sequencing Guide describes the effects of loading varying amounts of ligation libraries (SQK-LSK109) onto Oxford Nanopore MinION flow cells.

A constant molar ratio of adapted DNA library should be loaded onto the flow cell. Underloading will lead to pores in the single pore state and leading to reduced sequencing throughput. Overloading will also lead to reduced sequencing throughput. However, this molar ratio depends on the size distribution of the DNA and the ligation efficiencies.

In this study, we extracted HMW DNA from human whole blood, made 1 large LSK109 library, split the library, and loaded varying amounts of the same library onto three separate flow cells for sequencing. No size selection was performed on the DNA.

Study Synopsis

- Loading more library onto the flow cell increases throughput.
- Unexpected, loading more library onto the flow cell also decreased read length by enhancing bias towards short reads.
- For HMW DNA that has not been size selected, we recommend loading 300-500 ng of library onto the flow cell.
- For HMW DNA that has been size selected with SRE or SRE XL, we recommend loading 500-1000 ng of library onto the flow cell.

HMW DNA Extraction

Extraction Notes

- HMW DNA was extracted from human whole blood using the Nanobind CBB Big DNA Kit and HMW

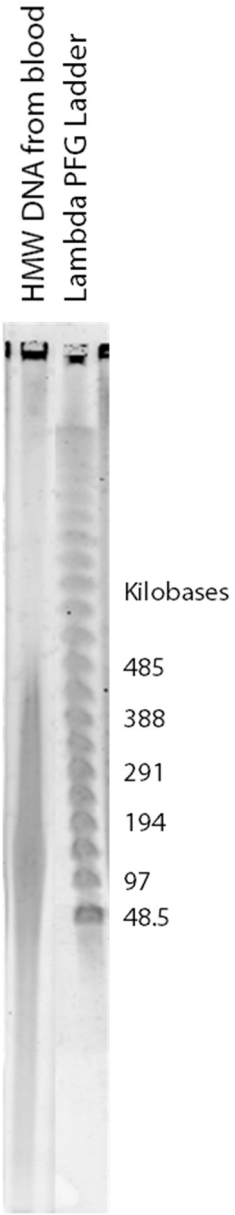
Extraction Results

- 200 µL of blood resulted in 4.1 µg of DNA with typical purity numbers

Sample Name	Sample Input	dsDNA (µg)	%RNA	A260/A280	A260/A230
Human Whole Blood	200 µL	4.1	19	1.91	1.62

DNA Size

- DNA size was 50 – 400 kb.



Oxford Nanopore GridION Sequencing

Library Preparation

- Single large library was prepared for sequencing using the Oxford Nanopore Ligation Sequencing Kit (LSK-109).
- 3 library preparations were performed in parallel following the Genomic DNA by Ligation (SQK-LSK109) protocol. Each prep used 1 µg of HMW DNA as input giving 3 µg total. The libraries were combined at the post-ligation AMPure step and eluted into one master library.
- Reaction times were increased by 6X. End-prep/repair at 20 ºC and 65 ºC was extended to 30 mins. Ligation time was extended to 60 min.
- AMPure elution times were extended to 10 mins.
- No size selection was performed

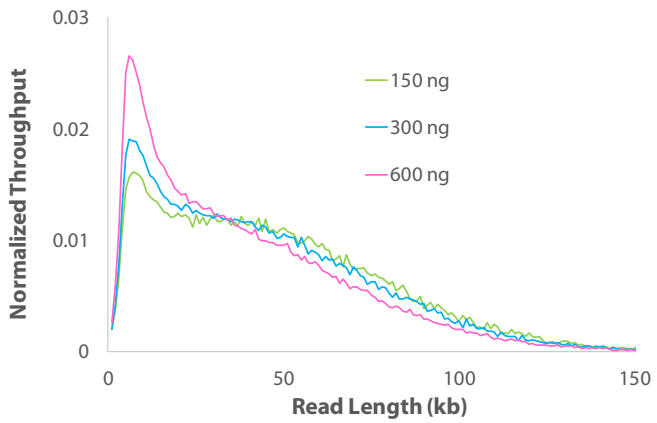
Sequencing

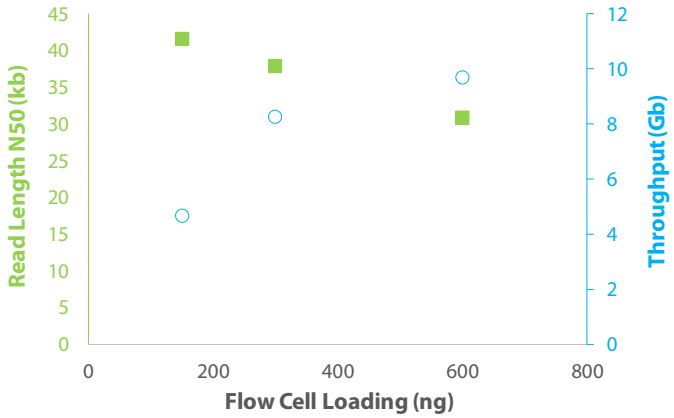
- 150, 300, and 600 ng of the same library was loaded onto 3 flow cells (FLO-MIN106D).
- All three samples were sequenced in parallel for 48 hours.
- No nuclease flush or refueling was performed.
- Fast basecalling was performed.

Sample	Library Prep Input	Final Library Amount	Flow Cell Loading
Human Whole Blood	3 µg	1.25 µg	150 ng / 300 ng / 600 ng

Sequencing Results

- Sequencing throughput increases with mass of DNA loaded
- Read length N50 decreases with mass of DNA loaded
- Increased loading disproportionately increases shorter reads





Human Whole Blood – ONT MinION/GridION Sequencing							
Sample	**Library Prep**	**Shear**	**Size Selection**	**Sequencing Time**	**Read Length N50 (bp)**	**Gb >100 kb**	**Total Data (Gb)**
150 ng Loaded	SQK-LSK109 FLO-MIN106D	5X Needle Shear	None	48 hr	41,608	0.3	4.7
300 ng Loaded	SQK-LSK109 FLO-MIN106D	5X Needle Shear	None	48 hr	37,854	0.4	8.3
600 ng Loaded	SQK-LSK109 FLO-MIN106D	5X Needle Shear	None	48 hr	30,845	0.4	9.7