Guidelines for Genomic DNA Sequencing in the PromethION (gDNA-ONT)

Introduction

Nanopore sequencing developed by Oxford Nanopore Technologies (ONT) uses molecular sensors or *nanopores* capable of measuring changes in electric current when a single DNA or RNA molecule passes through them. Different sequences going through a nanopore give different electrical signals, producing an electrical trace or 'squiggle.' These squiggles can be decoded into basecalls in real-time. Read lengths produced by Nanopore sequencing are only limited by the size of the library, which enables obtaining DNA reads spanning up to 2Mb. Nanopore technology can also sequence native DNA, allowing for the detection of base modifications. The PromethION sequencer is the highest throughput sequencer from ONT. Each PromethION flow cell is advertised to produce up to 180Gb[1] of data.

Genomic DNA (gDNA) sequencing applications supported by the PromethION include:

- ✓ Structural variation
- ✓ Assembly
- ✓ DNA base modifications
- ✓ Multiplex sequencing

Technical specifications

Advertised[1] flow cell performance for currently available flow cell types:

Table 1 ONT advertised flow cell specifications

Flow cell type	**R9.4.1**	**R10.3**
Yield per flow cell[1] DNA	Up to 180Gb	Up to 180Gb
1D accuracy	Up to 95%	Up to 95%
Q score (consensus)	~ Q42	~Q50
Recommended for	Higher output Consistent performance	Improved homopolymer performance Product in development. Performance may vary

[1]Performance may vary greatly depending on input material (DNA/cDNA/RNA), sample quality, library size, library purity, preparation methods. For details, consult the Nanopore website. **Please note:** these specifications are for guidance purposes only. The BRF does not guarantee these performance metrics will be met.

Genomic DNA processing at the BRF

The gDNA-ONT service offers sample QC, library preparation and sequencing of gDNA provided by clients. The workflow for this service is outline below:

Sample submission

Please complete the gDNA-ONT sample submission form in our online system BRF (see here for details on electronic submissions). The sample submission form asks for:

- ✓ Amount and quality of input material
- ✓ Research goal of your experiment
- ✓ Any QC data you have on your library (e.g. concentration readings and size traces)
- ✓ Additional procedures such as shearing, size selection, multiplexing.
- ✓ Acceptance of terms and conditions of service.

Once your samples have been submitted electronically, bring your samples in person or by courier to the BRF. Ensure samples are:

- Labelled appropriately: Write the *Sample Submission Id* and the *Request Id* from the electronic form. Label cap and side of the tube.
- Transported in conditions that preserve sample integrity (e.g tubes are in good condition, properly sealed, transported cold, proper padding to avoid crushing, etc)

Initial quality control checks (Initial QC)

Once received, we will check your sample against the following:

Table 2 gDNA QC parameters

QC Parameter	Specification	Measurement method
Appearance	Correct labelling and container integrity Clear viscous liquid with no visible particles of precipitates.	Visual check
Volume check	At least 50 ul per flow cell	Pipetting
Average fragment size	60Kb, no smearing below 15Kb	Pulse field electrophoresis (Femto Pulse)
Amount of material	5-10ug (100-300fmol) per flow cell	Qubit or equivalent
Purity	Absorbance ratios: 260/280: 1.8-2.0, 260/230: 2.0-2.2	Nanodrop

Project acceptance:

Upon QC completion, we will get in touch to discuss QC results and how to best proceed with sample processing. Your project will be marked as ***accepted*** when:

- Your samples meet QC parameters* as per table 2. Genomic DNA samples that fail QC are likely to perform poorly on a Nanopore flow cell.
- There is an agreement between you and the BRF on what services will be included (e.g. size selection, multiplexing, etc) and their price. A quote for the service can be accepted electronically via bookbrf.
- You have arranged enough electronic storage to transfer your data files to. We recommend ~1TB of disk per flow cell sequenced.

If samples in your project do not meet QC parameters, you can:

- Withdraw the project: you will only be charged the for the QC fees. Reagents/consumables purchased for your project may also be charged at the BRF's discretion.
- Resubmit your sample: you can reuse the sample submission form. QC for failed samples will still carry a fee.
- Proceed with the prep: Recommended only if it is apparent that your project goals can be met despite sample not meeting QC. The PI must provide explicit written authorisation for the project to proceed.

Library preparation and sequencing:

gDNA libraries will be prepared using the ONT ligation methods and protocols (SQK-LSK109). We also offer the following additional services:

Table 3 Additional services for gDNA preps

Additional service	Purpose
Sample clean-up with Ampure XP beads	May improve sample purity and ligation performance during prep.
Shearing using the Covaris gTube	To maximise flow cell output. N50s will be reduced
Short read elimination using the Circulomics SRE	Will improve N50.
Size selection using the Sage Blue Pippin	Will improve N50 and may reduce contaminants and impurities in the sample.
Barcoding using the EXP-NBD104, EXP-NBD114 kits	Will allow multiplexing and or running consecutive samples in the same flowcell

Whenever reasonable, we will test the library using a Flongle or MinION flowcell to rule out library performance issues prior loading the PromethION flow cell.

Your library will be sequenced in the PromethION using ONT standard methods.

Data collection:

We will perform fast real-time base calling for each flow cell as per ONT recommendations. Customers can choose to receive fast5 or fast5 *and* fastQ files. Boutique basecalling/analysis methods are not offered as part of the standard service.

Important: ONT sequencing is a fast growing and developing technology, and as such, hardware/reagents/consumables occasionally may not perform as advertised. ONT is also extremely sensitive to the quality and purity of the input material you provide. At the BRF, we will strive to give you the best possible output and data quality as per your project goals, but due to the above mentioned limitations, we are unable to guarantee data outputs or read quality for your runs.