Guidelines for client-made libraries for the PromethION Sequencer (ONT_Lib)

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 and full-length transcripts. Nanopore technology can sequence native DNA and RNA molecules, 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.
Sequencing applications supported by the PromethION include:

- ✓ Structural variation
- ✓ Assembly
- ✓ DNA and RNA base modifications
- ✓ Full length transcriptome
- ✓ Multiplex sequencing

Technical specifications:

Advertised[1] flow cell performance:

Flow cell type	**R9.4.1**	**R10.3**
Yield per flow cell[1] DNA/cDNA	Up to 180 Gb	Up to 180 Gb
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.

Supported sample/library types[2]:

Sample Type	**Single sample**	**Multiplex**
gDNA	Genomic DNA by Ligation (SQK-LSK109)	Native barcoding genomic DNA (with EXP-NBD104, EXP-NBD114, and SQK-LSK109)
RNA	Direct RNA sequencing (SQK-RNA002)	
cDNA-direct	Direct cDNA Sequencing (SQK-DCS109)	Direct cDNA Native Barcoding (SQK-DCS109 with EXP-NBD104 and EXP-NBD114)
cDNA-PCR	cDNA-PCR Sequencing (SQK-PCS109)	PCR-cDNA Barcoding (SQK-PCB109)

[2] R10.3 flow cells only support ligation kits (SQK-LSK109)

Processing your library at the BRF

Sample submission

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

- ✓ Prep kit type. *Only ONT prep kits and ONT supported 3rd party materials are recommended.*
- ✓ Amount and quality of input material
- ✓ Research goal of your experiment
- ✓ Conditions of library storage.
- ✓ Any QC data you have on your library (e.g. MinION run metrics, concentration readings and size traces)
- ✓ If a multiplexed library, the type of barcodes used

Once your samples have been submitted electronically, bring your samples in person or by courier to the BRF. Please ensure the following at the time of submission:

- ✓ Your libraries are labelled appropriately: Write the *Sample Submission Id* and the *Request Id* from the electronic form. Label cap and side of the tube.
- ✓ As required, you provide accessory run reagents.
- ✓ You handle/transport your libraries in a way that preserves integrity (e.g tubes are in good condition, properly sealed, transported cold, proper padding to avoid crushing, etc).

Initial quality control checks (Initial QC)

Prior running your library on a PromethION flow cell, we will perform the following quality control (QC) checks:

- ✓ Volume and appearance check
- ✓ Concentration quantification using the Qubit fluorometer, when applicable.
- ✓ Sizing profile using appropriate methods for the library type, when applicable (i. e. Bioanalyzer or Femto Pulse).
- ✓ We will assess the sequencing performance of your library on a Flongle or MInION flowcell.

Once we assess the quality of your library, we will report the results to you. You will be asked to:

- ✓ Decide if you wish to proceed with the PromethION run, based on the QC results
- ✓ Provide sufficient computer storage to copy your data to
- ✓ Provide accessory run reagents if required as per your prep type.

We will strive to give you the best possible output and data quality, but due to the newness of this technology we are unable to guarantee results for your run.

Data collection:

We will perform 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.

Data management and delivery

PromethION run outputs may exceed 1TB, which hinders out ability to provide long term data storage/back up for this service. To ensure a smooth and error-free data hand-over, we ask that data storage arrangements are made prior your sample is due to be sequenced.

- ✓ For clients with NCI accounts (ANU only): Transfer of data to an NCI account is the fastest and most reliable way to deliver data to you. If you are an ANU client, it is highly recommended you secure enough storage though an NCI project (nci.org.au). Please detail your NCI project and username in the electronic sample submission.
- ✓ For external clients: We will generate a transfer link using CloudStor. It is the client's responsibility to download the data and check its integrity.
- ✓ If you work in collaboration with an ANU bioinformatics team (e.g. the ABC or EMBL Australia), you are encouraged to negotiate NCI storage through your collaborators.

We require acknowledgement that you have received your data within 5 working days after hand over. Data will be archived by the BRF for a maximum of 3 months, and a you will be charged a weekly storage fee until you confirm your data has been received successfully. We reserve the right to permanently delete data after we receive confirmation of reception and/or after 3 months from the date of data hand over.
Storing and backing up your data is solely your responsibility. We strongly advise you back up your data securely upon reception. Options include:

- ✓ Mass Data Storage System (MSDD) through NCI can be suitable for data archiving.
- ✓ Cloud services such as AARNET CloudStor or AWS
- ✓ Online public data repositories (data can be under embargo for a period of time):
  - The Sequence Read Archive, SRA
  - European Nucleotide Archive, ENA
- ✓ Controlled-access data repositories:
  - European Genome-phenome Archive, EGA
  - Database of Genotype and Phenotype dbGAP

Note: Storing data in hard drives is discouraged as data transfer is slow and error prone.