Nanopore Sequencing Service Guide

Biomolecular Resource Facility (BRF)

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. Different sequences going through a nanopore give different electrical signals, producing a trace or 'squiggle' which 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 2 Mb. Nanopore technology can also sequence native DNA and RNA, allowing for the detection of base modifications. The PromethION sequencer is the highest throughput sequencer from ONT. A PromethION DNA Flow Cell typically produces 60-100 Gb per run.

Sequencing applications supported by the BRF's PromethION include:

- Sequencing of native DNA and RNA including base modifications
- Structural variation
- Assembly
- Full-length isoform

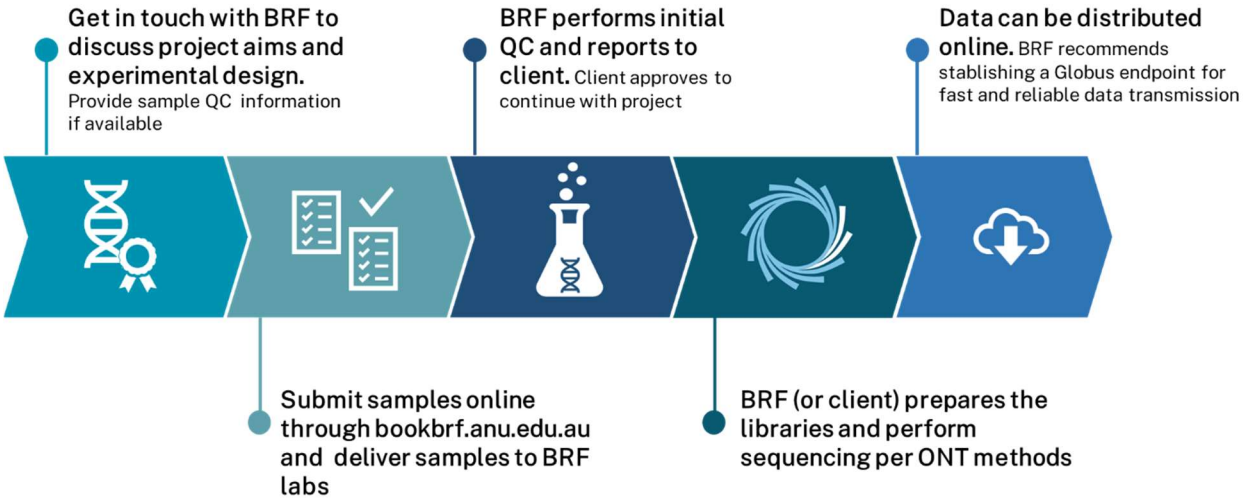
Nanopore service at the BRF

In addition to sequencing runs, the BRF offers comprehensive library preparation services. Our friendly and skilled staff are experienced and proficient in the handling and preparation of long-read sequencing libraries. BRF labs are equipped with a high throughput PromethION24 and all the necessary accessory equipment for the generation of high-quality ONT libraries. These include the Agilent Femto Pulse, the Sage Blue Pippin, the Agilent TapeStation and more. Our standard services include:

- DNA quality control checks including assessment of quantity, purity and integrity
- Pulse-field, gel based DNA size selection, short fragment depletion, bead purification
- Whole Genome Sequencing (WGS)
- Full length RNA sequencing from direct RNA and cDNA libraries
- Full length sequencing of amplicons and plasmids
- Sequencing of bacterial isolates

How to access BRF services

Our sample processing workflow is as follows:



Step 1: Discuss your project aims with the BRF

Please get in touch with the BRF early on your project to discuss your data needs. Information such as species, sample preservation method, extraction method, genome size, target N50, coverage requirements per sample etc. would help map the best strategy for your project.

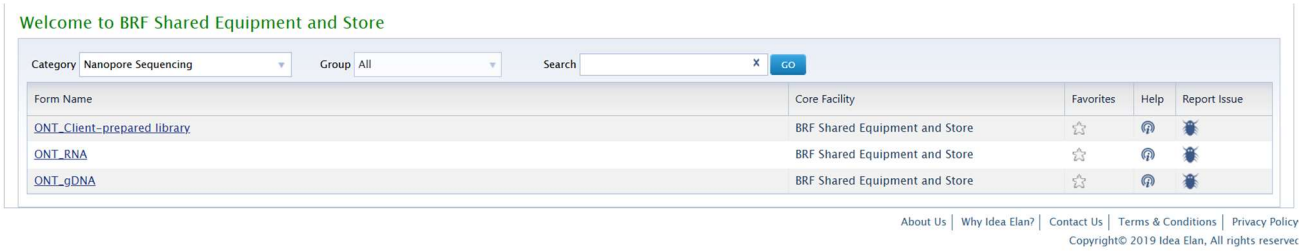
The quality of data and total output of a Nanopore run highly depends on the quality of the input material; hence, the most critical step for a long-read sequencing workflow is obtaining high-quality nucleic acid extractions. As this can be challenging for non-model organisms, it is highly recommended to:

- Use fresh or appropriately preserved biological specimens (e.g. snap frozen). Specimens preserved in ethanol, RNAlater or museum specimens often produce suboptimal DNA/RNA libraries.
- For WGS applications, choose extraction methods optimised to preserve high molecular weight (HMW) DNA. Commercial kits such as the Monarch® HMW DNA Extraction Kit from NEB or the PacBio Nanobind extraction kits can be used on a variety of sample types and starting materials. See Step 4 for quality requirements for WGS samples.

Step 2: Submit samples online using bookbrf

For first-time users: Please email LongRead.brf@anu.edu.au with your lab details and we will create an online account for the principal investigator (PI). When requesting your account, please provide details of the PI such as name, affiliation, work address, work email/phone number and other relevant billing information. We will let you know when the account is ready for access. PIs can log in and add additional lab members, or request BRF to add them.

Once your account has been created, please go to bookbrf (https://bookbrf.anu.edu.au/ANU/public/HomePage.aspx) and complete the sample submission form from the 'Submit Samples' from the 'Home Page':



Pick the sample submission that matches your project needs:

- ONT_Client-prepared library: For when you prepare your own library.
- ONT_RNA: For Direct RNA and cDNA PCR Nanopore prep from total RNA
- ONT_gDNA: For preps starting from genomic DNA

BRF also offers a full-plasmid sequencing service, with different sample requirements and submission guidelines. For details, please check our website:
Full Plasmid Sequencing:

When completing the appropriate form for your project, please note:

- In the 'Samples' section, the name you enter in the Sample ID* field is the name we will use to name your dataset.
- Please leave the "Add service" section blank as BRF fills it in at the end of the project.
- Please note that by submitting your project you agree with the BRF's terms and conditions of service.

When you start a form, it gets assigned a *Project ID* (e.g. ONT_gDNA10_566). This is a unique identifier for your project and all communications and associated data tracking will use this ID as reference.

Each sample in the project gets a BRF sample ID. This ID has the format: the last 3 digits of the Project ID, followed by "-" and the number of the sample in the order it was submitted. For the example above (ONT_gDNA10_**566**), if two samples are submitted their BRF sample IDs are **566**-1 and **566**-2, corresponding to the order they were submitted.

Step 3: Bring or send your samples to the BRF

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

- Submitted in Eppendorf 1.5mL lo-bind tubes (avoid other low-retention brands if possible). For submission exceeding 24 samples, please submit them in plates loaded by column and provide all relevant details in an excel spreadsheet.
- Ensure tubes are in good condition. Check that tubes are properly closed, sealed and with padding as needed to avoid crushing.
- Label tubes appropriately: Label cap and side of the tube with the BRF sample ID (e.g. 566-1). If there is enough space, include your sample name.
- Ensure samples are transported in conditions that preserve sample integrity. Bring or ship samples cold, minimizing freeze-thaw cycles. Ice or icepacks are usually appropriate for local or overnight delivery. Dry ice is preferred for extended shipping.

If dropping samples off in person, our location is:

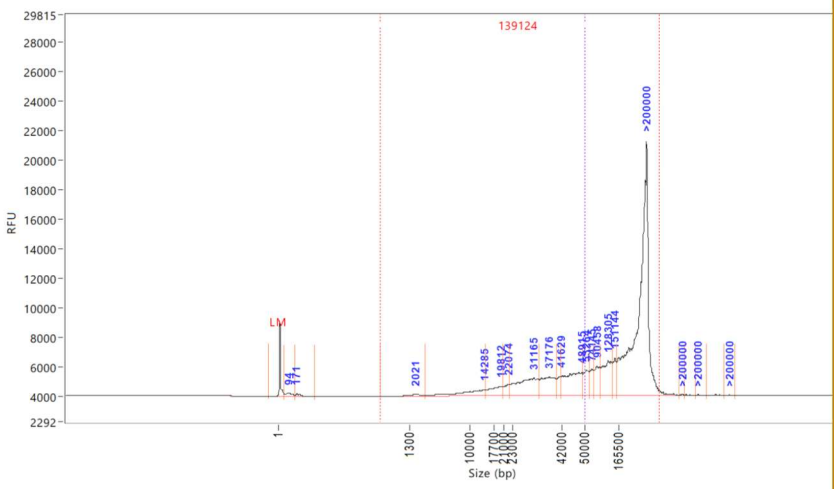
Level 2
The John Curtin School of Medical Research
Building 131, Garran Road
The Australian National University
Canberra ACT 2601
Ring extension x52397 (long-read) or x54326 (BRF general) and a member of our team will meet you.

For shipping, delivery details are:

Attention to BRF
The John Curtin School of Medical Research
Building 131, Floor Dock (Stores) off Garran Road
The Australian National University
Canberra ACT 2601

Step 4: BRF QC checks

Once we receive your samples, we will strive to perform full QC checks on your samples within 1 week of reception. QC criteria and specifications for a WGS sample are:

QC Criteria	Method	Specification
Appearance	Visual check	Clear viscous liquid with no visible particles or precipitates
Volume Check	Pipetting	At least 50 µL per flow cell
Fragment Size	Femto Pulse	Although Nanopore sequencing is agnostic to the DNA fragment distribution, projects aiming for very long reads (N50 > 30 Kb) require high integrity DNA as per example: 
Amount of Material	Qubit	For projects requiring very long reads or multiple flow cells, it is advised to submit 20 µg+ to perform DNA size selections and/or multiple library preparations. For projects without high N50 or data demands, 2 µg is usually suitable.
Purity	Nanodrop + Qubit	Absorbance ratios: 260/280: 1.8-2.0; 260/230: 2.0-2.2. Qubit/NanoDrop concentration > 0.5. Genomic DNA extractions have been RNase treated.

QC criteria and specifications for total RNA samples are:

QC Criteria	Method	Specification
Appearance	Visual check	Clear liquid with no visible particles or precipitates.
Volume Check	Pipetting	At least 10 µL per flow cell
RNA integrity	Bioanalyzer RNA Nano Chip or Tapestation RNA Screentape	RIN > 7.0
Amount of Material and Purity	Qubit	Recommended 1 µg per flow cell Absorbance ratios: 260/280: ~2.0; 260/230: 2.0-2.2.

We will report these measurements to you and discuss the suitability of your samples for ONT sequencing. If samples do not meet QC parameters, you can:

- Withdraw the project: you will only be charged for the QC fees, and reagents/consumables purchased on your behalf. Replacement samples should be submitted as a new project in bookbrf.
- Proceed with the prep: recommended only if it is apparent that your project goals can be met despite the sample not meeting QC. The client must provide explicit written

authorisation for the project to proceed and BRF makes no guarantees on output and/or data quality. Charges will apply for any failed procedures if authorised by the client.

Step 5: Library preparation and sequencing

BRF offers preparation of genomic libraries with standard ligation (SQK-LSK114), native barcoding (SQK-NBD114.24|96), and rapid barcoding (SQK-RBK114.24|96) and sequencing using FLO-PRO114M R10.4.1 flow cells.

RNA sequencing services include preparation of full-length cDNA libraries with SQK-PCB114.24 kit and FLO-PRO114M and direct RNA sequencing with SQK-RNA004 and FLO-PRO004RA flow cells.

In addition to standard library preparation methods, the BRF offers optional services that may improve the quality of starting material, depending on the project requirements. These include:

For DNA preps:

- AMPure XP bead clean-ups to improve sample purity
- Blue pippin size selection to improve DNA fragment distribution and sample purity
- Short read eliminator (SRE) to improve DNA fragment distribution
- DNA shearing with the Megaruptor 3 or the Hamilton Microlab to improve data output per flow cell

For RNA preps:

- RNAClean XP bead clean-ups to improve sample purity

BRF will inform clients of the performance of their libraries, and if required, will propose changes to the sequencing plans to suit the project's needs. Unless otherwise stated or requested, all sequencing runs are basecalled live using the super accuracy model inbuilt in the instrument control software at the time of sequencing.

Step 6: Data distribution

BRF will share the full run directory with the client. **Important note:** If you require raw data (pod5), run directories can exceed 1.5 TB for runs of ~100 Gb. Please ensure you have appropriate data storage arrangements at the beginning of the project.

For clients with NCI accounts, BRF can copy data directly into their allocation if temporary access is given to BRF technicians.

For other clients, the recommended method of data sharing is through the Globus app, which enables the delivery of datasets in a fast, secure and accurate manner. Some Australian universities and departments may have institutional access to Globus, so please check with your IT department about an institutional account. You can set up a Globus Personal Connect account and use it by mapping it to your own data cluster. If you require help with Globus, it is supported in Australia by AARNet.

If access to Globus is not possible, BRF can also provide data within Australia via downloadable link powered by AARNet FileSender,

Frequently asked questions

- *Does BRF provide DNA extraction service?*
  From 2025 BRF can offer extraction services from plant materials. This service is currently in development and due to the complexity and intrinsic variation in performance, outcomes are not guaranteed. Please get in touch with us at LongRead.brf@anu.edu.au if you want to know more.

- *BRF's QC shows that my DNA sample is fragmented, but the genomic TapeStation shows DNA that is highly intact. What result should I trust?*
  The Agilent Femto Pulse is the recommended method for DNA sizing for long-read sequencing as it separates DNA fragments using pulse field capillary electrophoresis. The TapeStation does not use Pulse field electrophoresis and hence it is not able to accurately discern large DNA fragments, often overestimating the quality of a DNA sample.

- *My sample did not generate enough data, or the quality/read length is not what I was hoping for. Do I get compensation, a refund or a free-of-charge repeat?*
  BRF does not offer compensation for failed/substandard sequencing results. On instances where there is a fault with ONT reagents and/or machine function and the vendor agrees to replace them, BRF will re-run them if there's enough library material to do so at no additional cost.

- *My sample did not meet QC requirements; do I have any options to proceed with prep and sequencing?*
  Depending on the quality assessment, the BRF can offer some optional procedures to improve the integrity and purity of a DNA sample, including AMPure XP bead clean-ups and Blue Pippin size selections. These methods may be suitable for samples where DNA mass is not limited, but case by case examination is required.

- *Does the BRF offer Bioinformatics services?*
  The BRF does not offer bioinformatics services. The ANU Bioinformatics consultancy works closely with the BRF and can offer services for a fee. Email: abc@anu.edu.au