

NGI OpenLab MinION Mk1D Instruction

This document is based on the [MinION Mk1D User Manual](#) from Oxford Nanopore Technologies (ONT). It contains hyperlinks (underlined in blue) that refer to relevant sections of the *MinION user manual*. Please read this document and the paragraphs the hyperlinks refer to, before attending the MinION sequencing introductory course.

Prior to the sequencing course, please consult the ONT website for detailed guidance on preparing your DNA or RNA, and how to prepare a library, under the [Documents](#) section.

1 Consumables and Equipment

Before starting a sequencing run, ensure that you have:

- Your library prepared according to instructions on the ONT website.
- The MinION device and USB 3.0 cable (provided by NGI OpenLab).
- A single-use flow cell (purchased separately by the user).
- Sequencing- and flow cell priming reagents included in the ONT library preparation kit. Please ensure that **both the flow cell** and the **reagents** are stored at their recommended temperatures until use!
- An SSD T9 drive for data transfer (provided by NGI OpenLab).

2 Safety

- Do not place the device on top of a laptop or other heat source.
- The storage buffer in the MinION flow cell (yellow colour) can release highly toxic gas if it comes in contact with acids. However, normal handling and storage do not pose any risk. Storage buffer is also present in the Wash Kit, used for washing and reloading flow cells. Use an additional pair of protective gloves when washing flow cells as an extra safety precaution. [Link to MSDS.](#)

3 Protocol

- 3.1.1 Sign in to the computer:
Sign in with the correct password.

3.1.2 Open the MinKNOW software and check connection to the MinION device.

3.1.3 Prepare the flow cell and perform a flow cell check

- Take out a flow cell and leave for 20 minutes at room temperature.
- Open the MinION device lid and slide the flow cell under the clip. Press down firmly on the priming port cover to ensure correct thermal and electrical contact. Close the lid and let the flow cell sit for 5-10 minutes to warm up.
- Perform a flow cell check according to the ONT instructions and make sure that the minimum number of pores are active (800 covered by warranty).

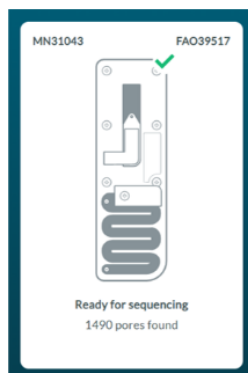


Figure 1. Flow cell ready for sequencing.

- If the flow cell does not have the expected number of active pores it is covered by a warranty, and the user should contact the Oxford Nanopore's support for assistance. Please note that for flow cells that you purchase yourself, any failed cell needs to be reported to ONT within 48 hours.

3.1.4 Loading of the sequencing library

In the [Document repository](#), please find your library preparation protocol (e.g. [ligation sequencing gDNA; SQK-MLK114.96-XL](#)) for instructions on how to prime the flow cell and load your DNA library. Each library preparation protocol includes recommendations for amount of library to load onto the flow cell.

The [NEB calculator is helpful](#) for calculating fmol when needed.

3.1.5 Starting a sequencing run

Refer to the [guide](#) on the ONT website on how to set up a sequencing run.

In short:

3.1.6 Navigate to the Start page and click **Start Sequencing** (Figure 2).

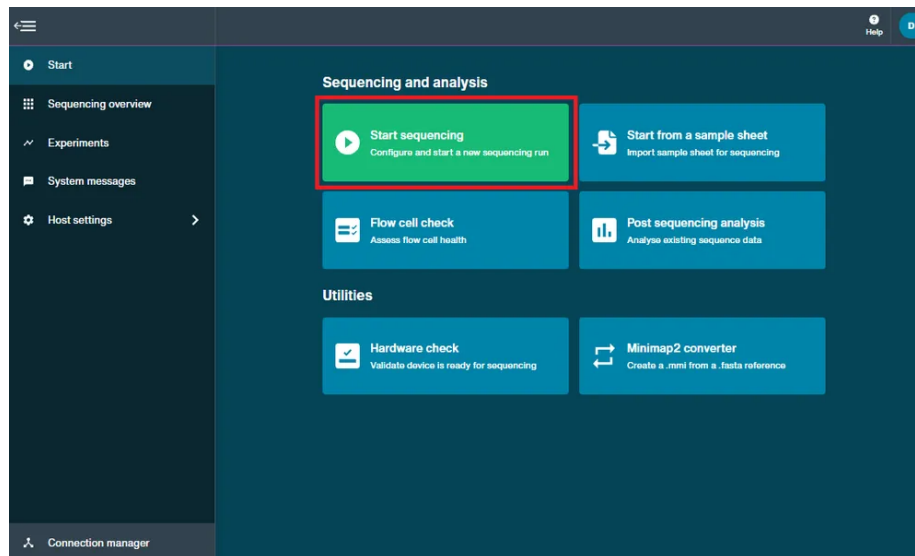


Figure 2.

- 3.1.7 In the Positions tab, fill in the experiment name, sample ID, and select the flow cell type. Click **Continue to kit selection**. Select the kit and any expansion(s) used to prepare the library. If barcodes have been used for multiplexed samples, choose your **barcoding** options.
- 3.1.8 Click **Continue to run options**. Keep the default settings unless there is a specific need to change them. We recommend adding pod5 as output. Click **Continue to analysis**.
- 3.1.9 Figure 3 shows the three options for basecalling and their recommended uses. To minimize the time the instrument is occupied, **real-time basecalling must be kept** as the selected setting.

There are three options for model selection in the drop down menu:

1. **High-accuracy basecalling (HAC)** - The HAC model is our default basecalling model and is recommended for most applications, including high-throughput projects focusing on variant analysis. It is able to keep up with a high-throughput sequencing experiment on a GridION or PromethION.
2. **Super-accurate basecalling (SUP)** - The SUP model is more computationally intensive than the HAC model and achieves the highest single-read accuracy. Recommended for de novo assembly projects and low-frequency variant analysis (e.g. somatic variation and single-cell applications).
3. **Fast basecalling** - This model is recommended for quick, real-time insights on sequencing data when compute resources are limited.

Figure 3.

3.1.10 Click **Continue to output**.

3.1.11 Specify your format and filtering options or keep the default settings.
Click **Continue to final review**.

3.1.12 Click **Start**.

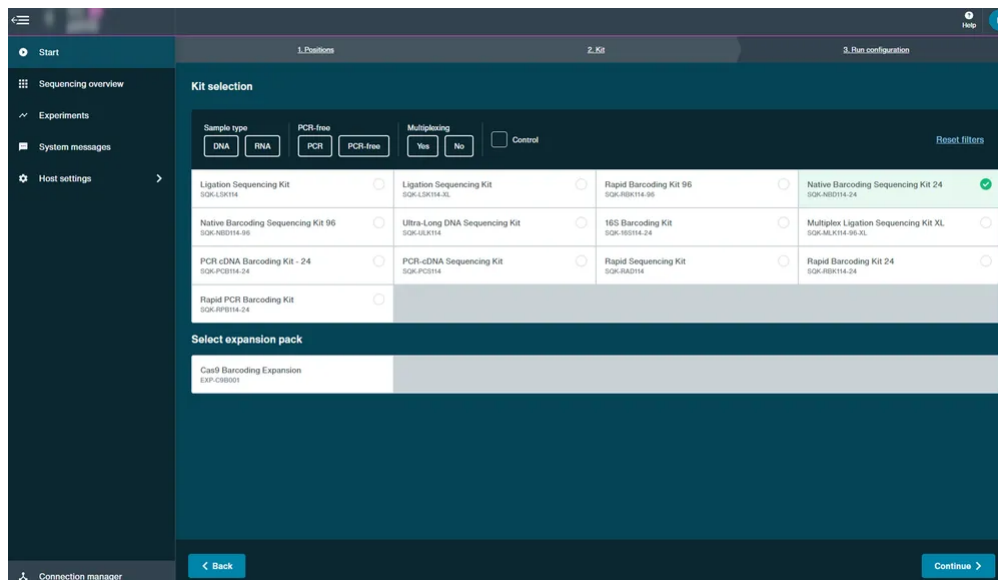


Figure 2

If **alignment** to a reference file is needed, or there is a need for **adaptive sampling**, please ask us in advance.

You can set up **data targets** or simply let the run go for the default 72 hours, according to the section called 'Monitoring and troubleshooting your sequencing run'.

4 Monitoring the run

Information about how to monitor your run can be found [here](#) (for monitoring the different run parameters) and [here](#) (for troubleshooting a poor run). The main parameters that are good to monitor are:

- The pore channel state, showing the channel states over time.
- The read length histogram.
- Cumulative output for data generation.
- Barcode read counts (if barcodes were used).
- Alignment hits (if alignment was selected).

To monitor the immediate health of the flow cell after starting a sequencing run, open the **channel states panel** or **pore activity plot** (Figure 5). Make sure that:

- The number of active pores reported in the initial Mux scan corresponds approximately to the numbers reported in the flow cell QC done prior to run start. If this is not the case, restart MinKNOW. If this does not help, restart the experiment or the computer. If the number of active pores remains low, a different flow cell should be used. Report to ONT.
- The number of available pores remain at an even level within the first 40 minutes of sequencing.



Figure 5. Light green: sequencing, Dark green: open pore, Dark blue: pore recovering, Light blue: pore inactive.

- **Pausing or refuelling** of the flow cell might be necessary depending on the experiment and library. Additional information on how to perform this procedure can be found [here](#).
- **Washing and reloading** If sequencing output is lower than expected, the Flow Cell Wash Kit (EXP-WSH004) can be used according to the recommended [wash protocol](#). Following the wash, either a new aliquot of the library can be loaded onto the flow cell, or the original library can be recovered from the flow cell and reloaded using [the library recovery procedure](#).

5 Cleanup and waste management

After the sequencing run is completed, please remove the used flow cell and put it in the designated box for used flow cells. All MinION flow cells must be returned to Oxford Nanopore after use (this will be managed by NGI OpenLab).

6 Retrieve data

6.1.1 When the run is complete, select **Export run report** and save the report temporarily to the desktop.

6.1.2 Connect the portable **SSD T9 drive** to the instrument computer using the supplied cable.

6.1.3 Open the terminal and enter:

```
1 cd /data/
```

6.1.4 Press **Enter**, then run the following script, where “XXXX” should be replaced by the name of your experiment:

```
1 rsync -rtv --progress XXXX /run/media/openlab/OpenLab/output/
```

6.1.5 To verify that all data has been successfully transferred to the SSD drive, run:

```
1 rsync -rtvc --progress XXXX /run/media/openlab/OpenLab/output/
```

6.1.6 No files should be listed between the lines **sending** and **sent**, indicating that all data has been transferred successfully:

```
1 sending incremental file list
2
3 sent ...
```

6.1.7 Finally, copy your Run report from the desktop to the SSD T9 drive by drag-and-drop.

6.1.8 When all data has been transferred, right-click on the SSD icon and choose “unmount” before removing the SSD T9 drive.

7 Returning the SSD T9 drive and deleting raw data from the instrument computer.

Once you have copied the raw data to your private computer, please return the used SSD T9 drive to the designated box in the NGI OpenLab.

Please note that raw data stored on the instrument computer is regularly removed by NGI staff to maintain sufficient disk space!

8 Support

Troubleshooting:

[Troubleshooting a run](#)

Technical support:

Contact NGI OpenLab staff first. If further assistance is required, contact technicalsupport@nanoporetech.com