Nanopore Protocol

MinKNOW

Computer requirements for MinKNOW

Version: MKE_1013_v1_revBZ_11Apr2016

Computer requirements

Computer specification

pdf
Uploaded file
MinION Mk1B Computer Requirements.pdf
pdf
Uploaded file
GridION Mk1 Computer Requirements.pdf
pdf
Uploaded file
PromethION Computer Requirements.pdf

Firewall settings needed to run the MinION Mk1B

During runs both MinKNOW and the Desktop Agent require access to the AWS IP ranges currently listed here for the region *eu-west-1*, and also access to:

178.79.175.200

96.126.99.215

Installing MinKNOW on Windows

~2 minutes

Download and installation

This part of the configuration will introduce the MinKNOW software and explain how to download and install it. There are three items to take particular note of:

- If anti-virus software stops the installation, contact your IT department
- The MinKNOW downloads as a .zip file and all files need to be extracted before proceeding with installation
- Data generated from the MinKNOW software needs to be stored on the SSD drive, and care should be taken to ensure the data location is set to the SSD during installation

If help is needed during installation, please refer to the **Support** area in the Community.

1 **Download MinKNOW**

- Download MinKNOW (MinION software) from the Downloads page on the Community
- Follow the on-screen instructions
- Contact the local IT administrators if there are any issues with permissions for installation

2 **Extract the .zip files.**

- Check that the Oxford Nanopore wheel icon is next to **MinKNOWInstaller_NC**
- Click on **MinKNOWInstaller_NC**

3 **Follow the installation prompts.**

4 **At the Installation Options screen, click 'Next' to accept default installation options.**

5 **In the Choose Data Location dialogue box, check that you are happy with the reads to change to the required SSD location and click on Install.**

In the Choose Data Location dialogue box, check that the read location is C:\data. If you prefer a different destination, enter the path in the box. Continue the download by clicking on Install.

Progression of installation

A dialogue box will open and shows progression of the installation.

6 **When installation is complete, click on 'Close' to see MinKNOW successfully installed.**

7 **Navigate to the MinKNOW icon on the desktop.**

> **END OF STEP**
>
> **The presence of the MinKNOW icon on the desktop indicates that this step has been successfully completed.**

Optional Action
If you have a proxy server and would like to set up MinKNOW using a proxy, follow the instructions below.

Open the `user_conf` file:

C:\ProgramFiles\OxfordNanopore\MinKNOW\conf\user_conf

And edit the following portion of the file:

```
"proxy": {
"cereal_class_version": 0,
"use_system_settings": true,
"auto_detect": true,
"auto_config_script": "",
"https_proxy": "",
"proxy_bypass": ""
```

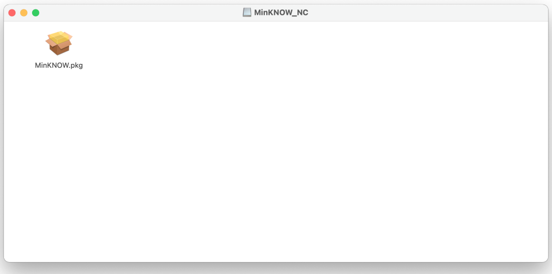
Edit the `https_proxy` setting, which should be in the style of:

scheme://[username:password@]host:port or "http://domain\\username:password@host:port", where "scheme" is one of https, socks, socks4 or socks5.

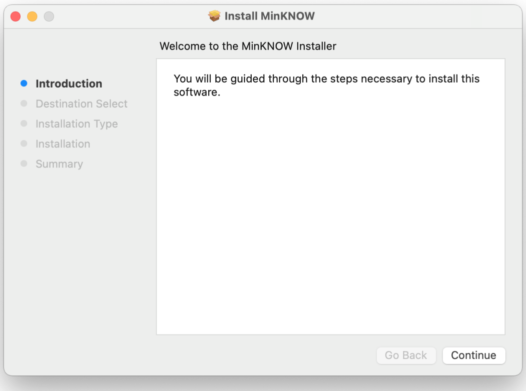
Installing MinKNOW on Mac OS X

1 Download MinKNOW

- Download MinKNOW (MinION Mk1B software) from the Downloads page on the Community.
- Once MinKNOW has downloaded, navigate to the folder in Applications and launch the MinKNOW.pkg installer.



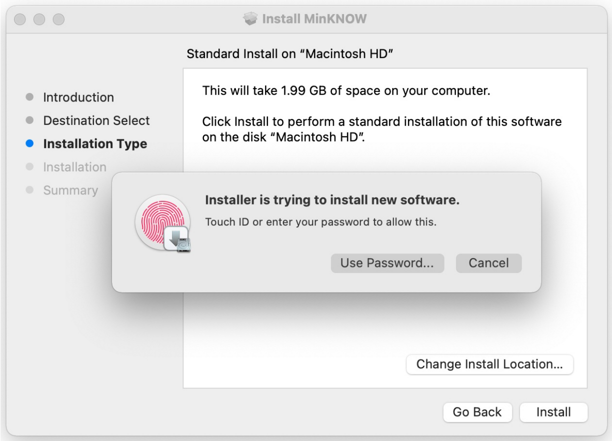
- Follow the on-screen instructions.



Welcome to the MinKNOW Installer

You will be guided through the steps necessary to install this software.

- Provide a fingerprint or password as required.



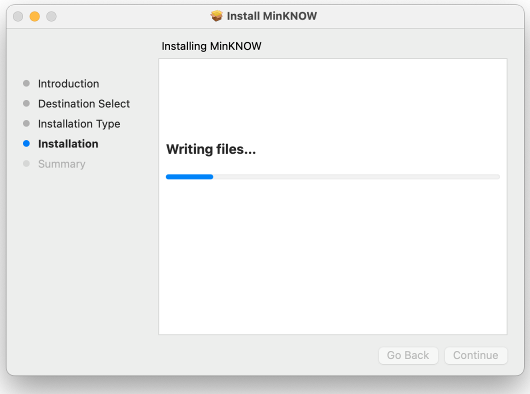
Standard Install on "Macintosh HD"

This will take 1.99 GB of space on your computer.

Click Install to perform a standard installation of this software on the disk "Macintosh HD".

Installer is trying to install new software.

Touch ID or enter your password to allow this.



Installing MinKNOW

Writing files...

- Once the installation is complete, exit the installer.

2 Navigate to the MinKNOW icon in the Applications folder.

> **END OF STEP**
>
> **The presence of the MinKNOW icon in the Applications folder indicates that this step has been successfully completed.**

Optional Action
Set the directory where the read files will be saved:

Open a command line and type:

```
sudo /Applications/MinKNOW.app/Contents/Resources/bin/config_editor --conf user --file /Applications/MinKNOW.app/Contents/Resources/conf/user_conf --set "output_dirs.base=/where the files wi
```

Add the full path to the location where you would like the read files saved in the place of "where the read files will be saved".

Optional Action
If you have a proxy server and would like to set up MinKNOW using a proxy, follow the instructions below.

Open the `user_conf` file:

/Applications/MinKNOW.app/Contents/Resources/conf/user_conf

And edit the following portion of the file:

```
"proxy": {
"cereal_class_version": 0,
"use_system_settings": true,
"auto_detect": true,
"auto_config_script": "",
"https_proxy": "",
"proxy_bypass": ""
```

Edit the **https_proxy** setting, which should be in the style of:

scheme://[username:password@]host:port or "http://domain\\username:password@host:port", where "scheme" is one of https, socks, socks4 or socks5.

Installing MinKNOW on Linux

1 **To add the Oxford Nanopore apt-get repository, run the command below on a terminal window:**

For Ubuntu 16:

```
sudo apt-get update
sudo apt-get install wget
wget -O- https://mirror.oxfordnanoportal.com/apt/ont-repo.pub | sudo apt-key add -
echo "deb http://mirror.oxfordnanoportal.com/apt xenial-stable non-free" | sudo tee /etc/apt/sources.list.d/nanoporetech.sources.list
```

For Ubuntu 18:

```
sudo apt-get update
sudo apt-get install wget
wget -O- https://mirror.oxfordnanoportal.com/apt/ont-repo.pub | sudo apt-key add -
echo "deb http://mirror.oxfordnanoportal.com/apt bionic-stable non-free" | sudo tee /etc/apt/sources.list.d/nanoporetech.sources.list
```

For Ubuntu 20:

```
sudo apt-get update
sudo apt-get install wget
wget -O- https://mirror.oxfordnanoportal.com/apt/ont-repo.pub | sudo apt-key add -
echo "deb http://mirror.oxfordnanoportal.com/apt focal-stable non-free" | sudo tee /etc/apt/sources.list.d/nanoporetech.sources.list
```

2 **Install MinKNOW using the command:**

```
sudo apt-get update
sudo apt-get install minion-nc
```

3 **Open the MinKNOW GUI from the applications list.**

Default installation directories

For the MinKNOW software:
`/opt/ont/minknow`

For the MinKNOW user interface:
`/opt/ont/minknow-ui`

Location of the reads folder:

The reads folder is in `/var/lib/minknow/data`

Location of the log files:

The MinKNOW logs are located in `/var/log/minknow`
The Guppy basecaller logs are located in `/var/log/minknow/guppy`

Optional Action
If you have a proxy server and would like to set up MinKNOW using a proxy, follow the instructions below.

Open the `user_conf` file:

/opt/ONT/MinKNOW/conf/user_conf

And edit the following portion of the file:

```
"proxy": {
"cereal_class_version": 0,
"use_system_settings": true,
"auto_detect": true,
"auto_config_script": "",
"https_proxy": "",
"proxy_bypass": ""
```

Edit the `https_proxy` setting, which should be in the style of:

scheme://[username:password@]host:port or "http://domain\\username:password@host:port", where "scheme" is one of https, socks, socks4 or socks5.

Installing a GPU version of Guppy with MinKNOW for MinION on Linux

Installing a GPU version of Guppy with MinKNOW for MinION on Linux

Version: MKE_1013_v1_revBZ_11Apr2016

> IMPORTANT
>
> **The GPU version of Guppy must be installed on the same system to which the MinION is connected.**
>
> GPU basecalling is supported on NVIDIA GPUs only, and only on Linux and Windows.
> The installation of the GPU is done at the user's own risk. Misconfiguration of the GPU may result in slow basecalling and/or a large number of skipped reads if the basecall server crashes due to misparameterisation.
>
> A GPU with at least 8 GB of memory is recommended. GPUs with less than 4 GB of memory may not work.

The following commands need to be entered into a terminal. Note that some of them will require superuser privileges:

1 **Identify the version of the Guppy basecall server that MinKNOW is using:**

```
/usr/bin/guppy_basecall_server --version
```

2 **Download the archive version of GPU-enabled Guppy from the Nanopore Community.**

The specific URL to use is:

```
https://mirror.oxfordnanoportal.com/software/analysis/ont-guppy_<version>_linux64.tar.gz
```

Where `<version>` is the numeric part (major.minor.patch) obtained from the step above.

Example:
https://mirror.oxfordnanoportal.com/software/analysis/ont-guppy_3.2.10_linux64.tar.gz

Note that the version of Guppy **must** match the version packaged within MinKNOW to prevent errors. The exact version to use can be obtained from the output of Step 1 or from the MinKNOW release notes.

3 **Extract the archive to a folder.**

Example:

```
tar -C /home/myuser/ont-guppy -xf ont-guppy_XXX_linux64.tar.gz
```

4 **Make sure the archive version of Guppy runs on your machine.**

Note: see the installation section of the Guppy protocol. For example, you may need to install GPU drivers.

5 **Rename the existing override.conf file so that it does not override our new settings:**

```
sudo mv /etc/systemd/system/guppyd.service.d/override.conf /etc/systemd/system/guppyd.service.d/override.conf.old
```

6 **Use systemctl to edit the existing guppyd service (this will open a text editor with a copy of the existing service file):**

```
sudo systemctl edit guppyd.service --full
```

7 **Edit that new service file to point to your GPU version of Guppy, and add the appropriate device flag. You can change any other server arguments at the same time.**

For example, change this line in the service file:

```
ExecStart=/opt/ont/guppy/bin/guppy_basecall_server <things>
```

...to this (make sure you retain the "--port" argument exactly as it used to be -- this is how MinKNOW communicates with the basecall server):

```
ExecStart=/home/myuser/ont-guppy/bin/guppy_basecall_server <things> -x cuda:all
```

8 **Save the file and exit the text editor (the filename may look odd, but systemctl should change it to the correct name later).**

9 **Stop the MinKNOW service:**

```
sudo service minknow stop
```

10 **Stop the guppyd service:**

```
sudo service guppyd stop
```

11 Confirm the guppy_basecall_server process is not running:

```
$ ps -A | grep guppy_basecall_
```

If the result of the above command is not blank, manually kill the process:

```
sudo killall guppy_basecall_server
```

12 Start the guppyd service:

```
sudo service guppyd start
```

13 Confirm the guppy_basecall_server is running and is using the GPU:

```
nvidia-smi
```

Optional Action
If the guppy_basecall_server is not launching correctly, check its log output using journalctl ("-n 100" shows the last 100 entries in the journal) to see what is going wrong:

```
sudo journalctl -u guppyd.service -n 100
```

14 Start the MinKNOW service:

```
sudo service minknow start
```

15 Monitor your first sequencing run using the MinKNOW GUI to make sure basecalling is working as expected.

Troubleshooting

If some part of the above process does not work, then it is possible the guppyd service may end up misconfigured, and may be automatically disabled by the system. There are a few diagnostic checks that can be performed.

16 Check the Guppy basecall server logs.

Guppy log files are stored in `/var/log/guppy`

17 Use journalctl to directly read the log entries produced by guppy and systemctl:

```
sudo journalctl -u guppyd.service -n 100
```

18 Check whether the service is enabled.

```
systemctl list-unit-files | grep guppyd.service
```

If the service is not listed as "enabled", then it will either be marked as "disabled" or "masked". You can reset those statuses as described below.

If the service is marked as "disabled":

```
sudo systemctl enable guppyd.service
```

If the service is marked as "masked":

```
sudo systemctl unmask guppyd.service
```

You may then need to enable the service as described above.

19 Reinstall the service.

```
sudo apt install --reinstall ont-guppyd-for-minion
sudo systemctl revert guppyd.service
sudo service guppyd restart
```

You will then need to repeat the above step to rename the guppyd override.conf file.

Setting GPU parameters for lower-memory graphics cards.

When using GPUs with 8 GB of memory or less, larger basecall models (such as HAC and Sup) may not run. In this case it is recommended to lower the `chunks_per_runner` parameter of the basecall server to reduce memory use. This parameter is set when launching the basecall server.

Edit the guppyd service file and add `--chunks_per_runner <value>` to the `ExecStart` line, before restarting the service.

The following settings are recommended for 8 GB graphics cards. For cards with less GPU memory, or if the GPU is being used by other processes, these numbers may need to be lowered.

- For HAC or modified basecalling models, use `--chunks_per_runner 160`
- For Sup basecalling models, use `--chunks_per_runner 10`

Installing a GPU version of Guppy with MinKNOW for MinION on Windows

> **IMPORTANT**
>
> **The GPU version of Guppy must be installed on the same system to which the MinION is connected.**
>
> GPU basecalling is supported on NVIDIA GPUs only, and only on Linux and Windows.
> The installation of the GPU is done at the user's own risk. Misconfiguration of the GPU may result in slow basecalling and/or a large number of skipped reads if the basecall server crashes due to misparameterisation.
>
> A GPU with at least 8 GB of memory is recommended. GPUs with less than 4 GB of memory may not work.

The following commands need to be entered into a Windows command prompt which has been run as an adminstrator. They also assume a standard MinKNOW installation, where the location of MinKNOW is C:\Program Files\OxfordNanopore\MinKNOW.

1 **Identify the version of the Guppy basecall server that MinKNOW is using.**

```
"C:\Program Files\OxfordNanopore\MinKNOW\guppy\bin\guppy_basecall_server.exe" --version
```

2 **Download the archive version of GPU-enabled Guppy from the Nanopore Community. The specific URL to use is:**

```
https://mirror.oxfordnanoportal.com/software/analysis/ont-guppy-<version>-win64.msi
```

Where `<version>` is the numeric part (major.minor.patch) obtained from the step above.

For example:

```
https://mirror.oxfordnanoportal.com/software/analysis/ont-guppy-3.2.10-win64.msi
```

Note that the version of Guppy **must** match the version packaged within MinKNOW to prevent errors. The exact version to use can be obtained from the output of Step 1 or from the MinKNOW release notes.

3 **Install the .msi from step 2, and note the location to which you installed Guppy.**

For example, you may have chosen to install Guppy in `C:\Program Files\OxfordNanopore\ont-guppy`.

4 **Make sure that this version of Guppy runs on your machine.**

Note: see the installation section of the Guppy protocol. For example, you may need to install GPU drivers.

5 **Modify MinKNOW's application configuration to enable GPU basecalling and set the appropriate settings.**

Where `C:\Program Files\OxfordNanopore\ont-guppy` is the location Guppy was installed to in step 3 above:

```
"C:\Program Files\OxfordNanopore\MinKNOW\bin\config_editor.exe" ^
--conf application --filename "C:\Program Files\OxfordNanopore\MinKNOW\conf\app_conf" ^
--set guppy.server_config.server_executable="C:\Program Files\OxfordNanopore\ont-guppy\bin\guppy_basecall_server.exe" ^
--set guppy.client_executable="C:\Program Files\OxfordNanopore\ont-guppy\bin\guppy_basecall_client.exe" ^
--set guppy.server_congif.gpu_calling=1 ^
--set guppy.server_config.num_threads=3 ^
--set guppy.server_config.ipc_threads=2
```

Information about Guppy settings can be found in the appropriate section of the Guppy protocol.

6 **Restart the MinKNOW service:**

1. Open the Windows menu.
2. Type "services" and select the **Services** app that is displayed.
3. Scroll down and find the **MinKNOW** service.
4. Right-click on it and select **Restart**.

7 **Confirm the guppy_basecall_server is using the GPU:**

```
nvidia-smi
```

8 **Monitor your first sequencing run using the MinKNOW GUI to make sure basecalling is working as expected.**

Troubleshooting

If step 8 above does not show guppy_basecall_server using the GPU, or if Guppy crashes frequently, then it is recommended to check the Guppy log files. These files are normally found in `C:\data\guppy_logs`, and a new file will be created every time the basecall server is launched.

If there is no server log with a timestamp that roughly matches step 6 ("Restart the MinKNOW service") above, then a new basecall server has not been launched. In this case, restart the MinKNOW service. If there is still no new log file created, restart your computer.

If there is a new server log file but it does not contain the parameters that were set as part of step 5 ("Modify MinKNOW's application configuration") above, then repeat steps 5 and 6.

If, during step 5, you see the following error message:

```
Failed to open C:\Program Files\OxfordNanopore\MinKNOW\conf\app_conf for writing.
```

Then your terminal has not been run as an administrator.

Setting GPU parameters for lower-memory graphics cards.

When using GPUs with 8 GB of memory or less, larger basecall models (such as HAC and Sup) may not run. In this case it is recommended to lower the `chunks_per_runner` parameter of the basecall server to reduce memory use. This parameter is set when launching the basecall server.

Edit MinKNOW's application configuration to add the `--chunks_per_runner` option to Guppy's "extra arguments" section:

```
"C:\Program Files\OxfordNanopore\MinKNOW\bin\config_editor" ^
--conf application --filename "C:\Program Files\OxfordNanopore\MinKNOW\conf\app_conf" ^
--set guppy.extra_arguments="--chunks_per_runner <value>"
```

Or `--set guppy.extra_arguments=""` to undo your changes.

The following settings are recommended for 8 GB graphics cards. For cards with less GPU memory, or if the GPU is being used by other processes, these numbers may need to be lowered.

- For HAC or modified basecalling models, use `--chunks_per_runner 160`
- For Sup basecalling models, use `--chunks_per_runner 10`

MinKNOW updates for Windows and Mac OS X

MinKNOW updates

Availability of updates to the MinKNOW software are indicated via the MinKNOW host settings or as a pop-up when first opening the software. The user should follow the on-screen instructions to install the new versions.

The details of the update will be communicated in Nanopore Community announcements.

We urge users to update as soon as reasonably possible after the release has been made available.

> **IMPORTANT**
>
> **Securing custom scripts prior to updating MinKNOW**
>
> If you have created custom scripts in MinKNOW, care should be taken to store them securely so they are not overwritten during software updates.
>
> Please also be aware that the script structure can change between MinKNOW versions; custom scripts written for one version may not be compatible with the next.

1 **Open the MinKNOW GUI via the desktop icon and the auto-updater will automatically check for updates when connected to the device.**

2 **A dialogue box will open when a new update is available.**

Select **Get Update** to update the device software automatically.

Updates may be skipped. However, we recommend to **update the device as soon as updates are available.** Some updates will be mandatory to use the device and unable to be skipped.

Update available

Do you wish to install MinKNOW 20.c.36?

Current Version
MinKNOW 20.b.40

New Version
MinKNOW 20.c.36

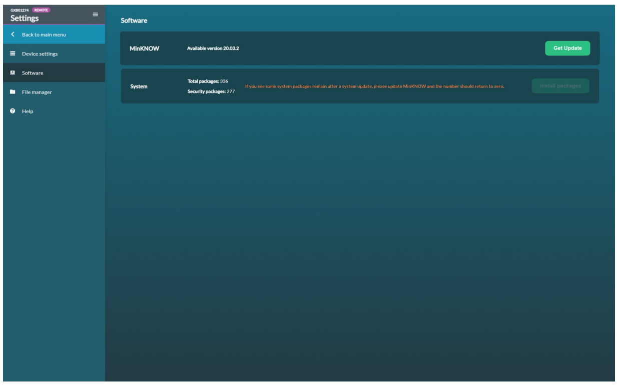
Note: For MinION Mk1B, clicking 'Get Update' will open the Software Downloads page on the connected computer for the user to download the updated MinION software.

Users are able to update the device from the 'Software' section of the Host settings.

3 **Navigate to 'Software' in the Host settings.**

An update button will appear when a new version of the software is available to download.

Select **Get Update** to open the installer window.



MinKNOW Available version 20.03.2

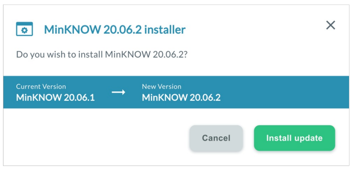
System Total packages: 336 Security packages: 277

If you see some system packages remain after a system update, please update MinKNOW and the number should return to zero.

Note: For MinION Mk1B devices, clicking 'Get Update' will open the Software Downloads page on the connected computer for users to select and download the updated MinION software.

4 **Click 'Install Update' in the installer window to download the new update.**

The changelog for the new updates will be displayed in this dialogue box.

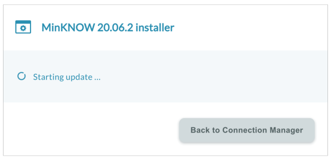


Do you wish to install MinKNOW 20.06.2?

Current Version
MinKNOW 20.06.1 → New Version
MinKNOW 20.06.2

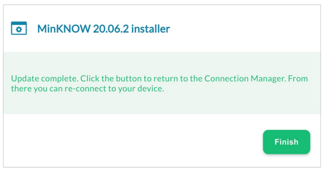
5 **Wait for the software to update.**

Note: This may take over several minutes to install. The device will **Reboot** once the update has installed.



Starting update ...

6 **The installer window will notify the user when the update is complete.**



Update complete. Click the button to return to the Connection Manager. From there you can re-connect to your device.

IMPORTANT

Re-installing MinKNOW

In some cases (e.g. if there is a problem with the version of MinKNOW that you are using), it may be necessary to re-install the software. To do this, you first need to do a full uninstall of the current version. To do this, follow the instructions in the Support article: How do I do a full uninstall of MinKNOW?

MinKNOW updates for Linux

1 **To update MinKNOW to a new version:**

```
sudo apt-get update
sudo apt-get install minion-nc
```

The latest version of MinKNOW will be installed.

IMPORTANT

Re-installing MinKNOW

In some cases (e.g. if there is a problem with the version of MinKNOW that you are using), it may be necessary to re-install the software. To do this, you first need to do a full uninstall of the current version. To do this, follow the instructions in the Support article: How do I do a full uninstall of MinKNOW?

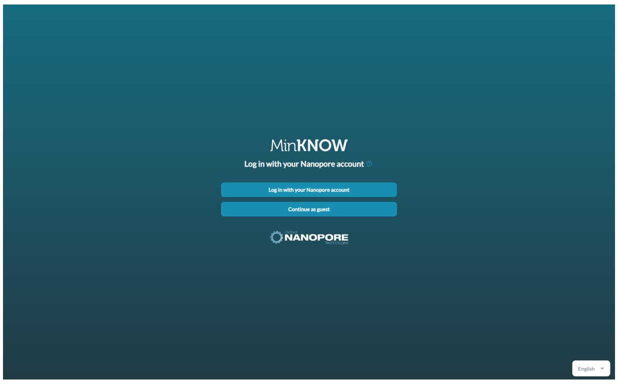
Login and logout

1 **We recommend users to log into the MinKNOW software using their Community credentials.**

If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

To log in, you must be connected to the internet.

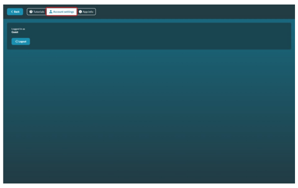
From here, users may also alter language in the user interface in the bottom right corner.



2 **To logout of the MinKNOW software:**

1. Select the button in the top right corner, labelled with your initials or 'Guest' and click 'Logout'.

1. Navigate to Application settings by clicking 'Application settings' and opening the 'Account settings' tab.

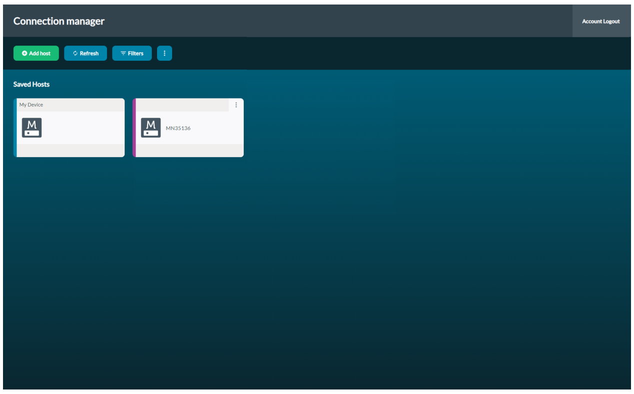


Connection Manager

The Connection Manager enables the user to connect with their devices.

MinKNOW can be either directly or remotely connected to a device.

- Blue tag: direct
- Purple tag: remote

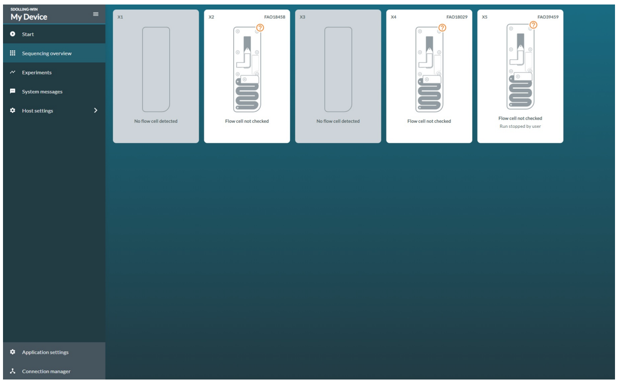


1 **An icon of the connected sequencing device will appear on the Connection Manager. Select the device icon to connect to your sequencing device.**

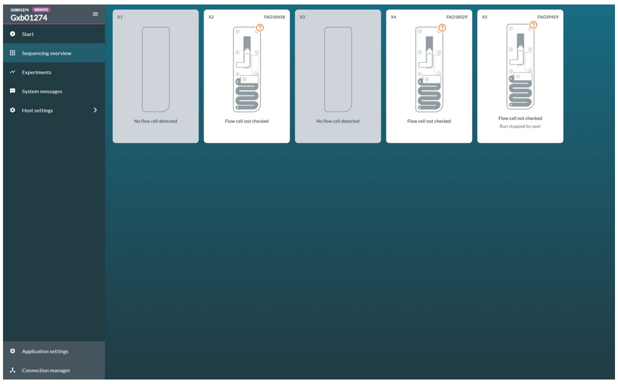
2 **The user will be navigated to the Sequencing Overview page of the device.**

The flow cell state will be displayed here.

GridION device with direct connection:



GridION device with remote connection:



The user is now able to navigate through MinKNOW to start an experiment or post-run analysis.

Device security settings

Default security settings

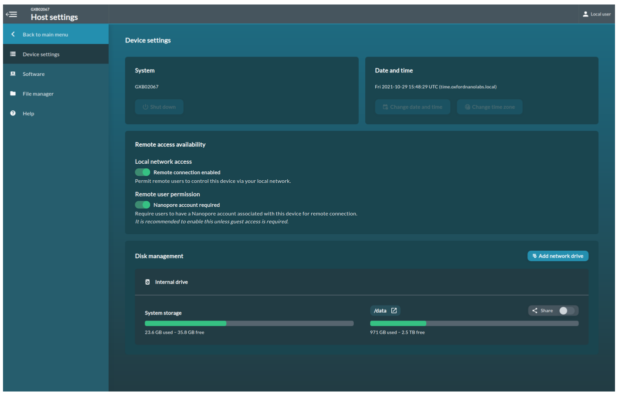
Default security settings for devices requires users to have a Nanopore account to remotely connect to a device.

To change the remote access availability, settings can be updated when directly connected to a device.

1 **Open 'Host settings' when directly connected to a device.**

2 **Navigate to the 'Device settings' and security settings options are located in 'Remote access availability'.**

Select options for local and remote access.



Device settings

System

GXB02067

Date and time

Fri 2021-10-29 15:48:29 UTC (time.oxfordnanolabs.local)

Remote access availability

Local network access

Remote connection enabled

Permit remote users to control this device via your local network.

Remote user permission

Nanopore account required

Require users to have a Nanopore account associated with this device for remote connection.
It is recommended to enable this unless guest access is required.

Disk management

Internal drive

System storage

23.6 GB used – 35.8 GB free

/data

971 GB used – 2.5 TB free

Homepage

Homepage overview

The MinKNOW Homepage enables the user to navigate to:

a. **Start** homepage

b. **Sequencing Overview** of connected flow cells

c. Recent and current **Experiments**

d. **System Messages**

e. **Host Settings**

f. **Application Settings** contain tutorials, account/login settings and MinKNOW GUI information

g. **Connection Manager** to connect with other available devices

h. **Start Sequencing** experiment

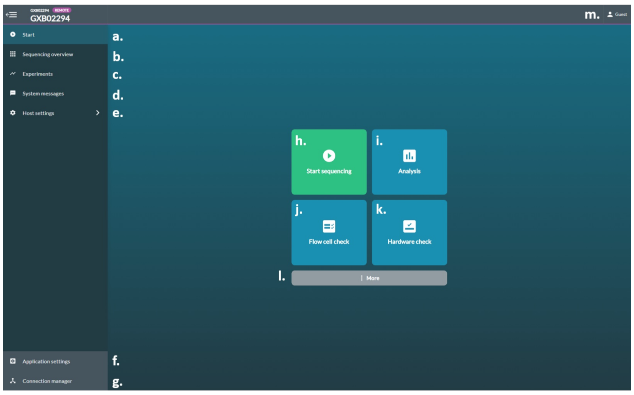
i. Post-run **Analysis**

j. **Flow Cell Check**

k. **Hardware Check**

l. **More** includes option to generate .mmi from .fasta file or to import a sample sheet

m. **Guest/initials** to logout

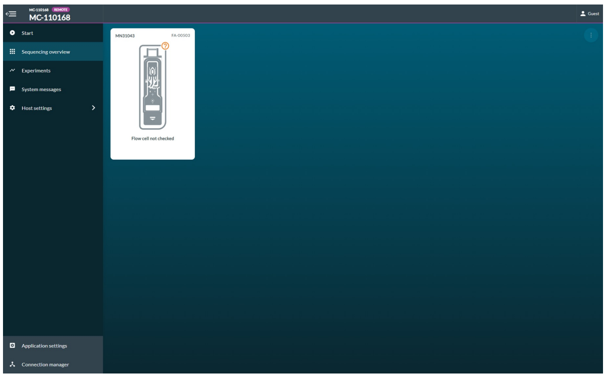


Sequencing Overview

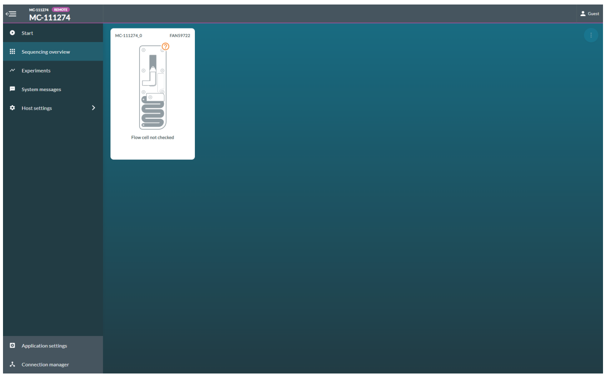
This page displays the inserted flow cell state and progress of a current sequencing experiment, including pausing, MUX scan and basecalling.

Flow cells with **no checks**:

- Flongle:



- MinION:

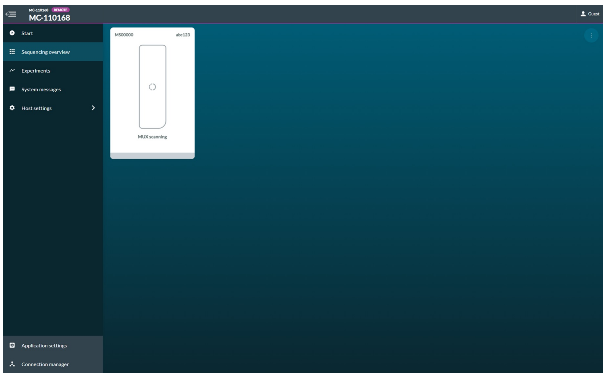


- GridION:

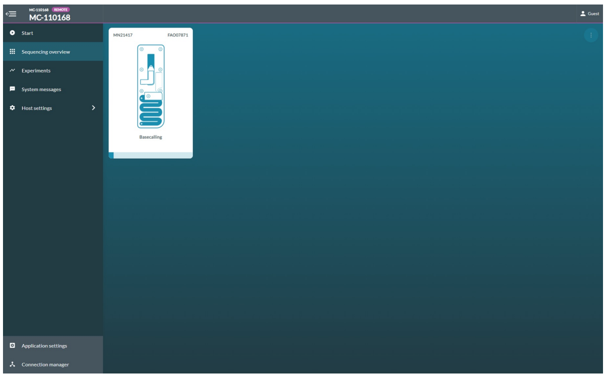
- PromethION:

Sequencing states on Flongle/MinION:

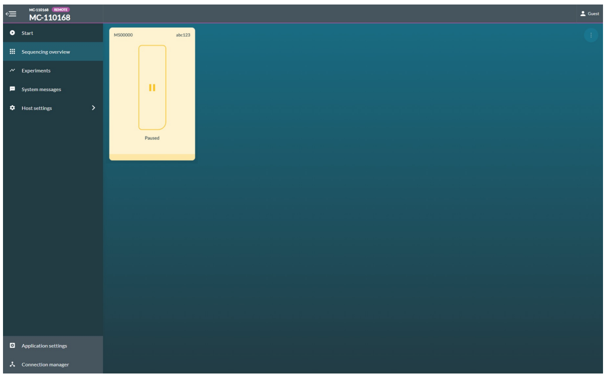
- MUX scanning:



- Basecalling catch up:

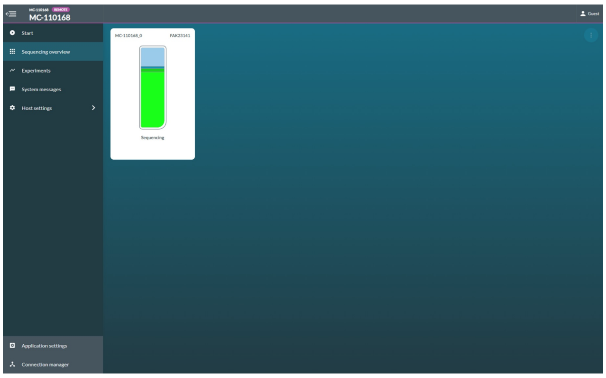


- Run paused:



Flow cell health will be displayed when sequencing after the first Mux scan.

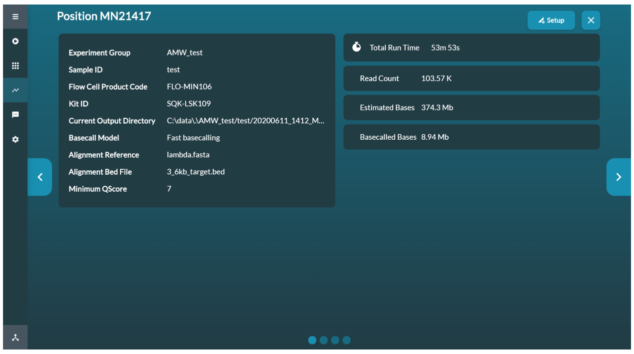
- Flongle/MinION:



- GridION:

- PromethION:

Select the flow cell to open the **quick view** of the current experiment.



Experiment Group	AMW_test
Sample ID	test
Flow Cell Product Code	FLO-MIN106
Kit ID	SQK-LSK109
Current Output Directory	C:\data\\AMW_test/test/20200611_1412_M...
Basecall Model	Fast basecalling
Alignment Reference	lambda.fasta
Alignment Bed File	3_6kb_target.bed
Minimum QScore	7

Total Run Time 53m 53s

Read Count 103.57 K

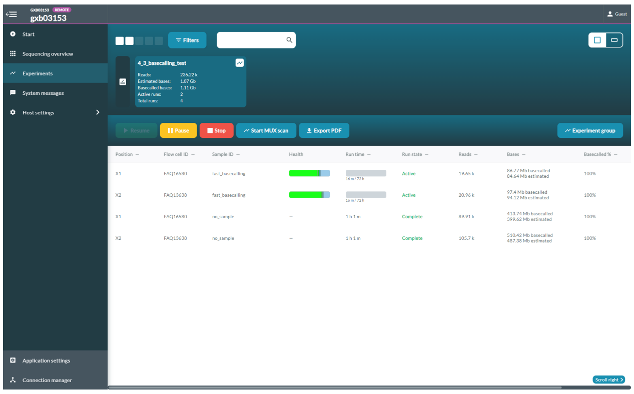
Estimated Bases 374.3 Mb

Basecalled Bases 8.94 Mb

Experiments page overview

The experiments page displays summary information for all experiments and device check data. The user is able to control runs and see real-time information from sequencing flow cells.

All previous runs can be viewed here until MinKNOW service is restarted (e.g. after a sequencing device reboot).

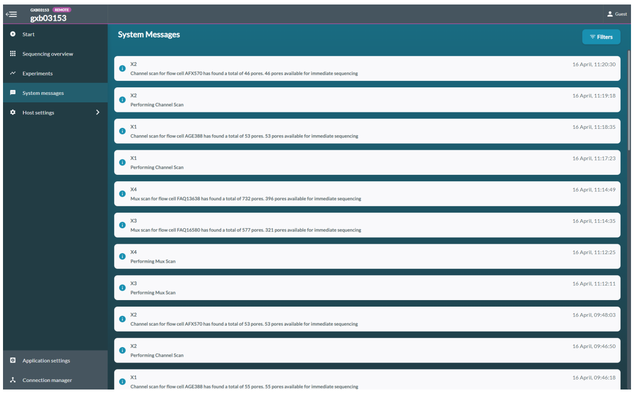


4_3_basecalling_test
Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%
X2	FAQ13638	fast_basecalling		16 m / 72 h	Active	20.96 k	97.4 Mb basecalled 94.12 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

System Messages

All device reports and messages are displayed here.



X2 — 16 April, 11:20:30
Channel scan for flow cell AFX570 has found a total of 46 pores. 46 pores available for immediate sequencing

X2 — 16 April, 11:19:18
Performing Channel Scan

X1 — 16 April, 11:18:35
Channel scan for flow cell AGE388 has found a total of 53 pores. 53 pores available for immediate sequencing

X1 — 16 April, 11:17:23
Performing Channel Scan

X4 — 16 April, 11:14:49
Mux scan for flow cell FAQ13638 has found a total of 732 pores. 396 pores available for immediate sequencing

X3 — 16 April, 11:14:35
Mux scan for flow cell FAQ16580 has found a total of 577 pores. 321 pores available for immediate sequencing

X4 — 16 April, 11:12:25
Performing Mux Scan

X3 — 16 April, 11:12:11
Performing Mux Scan

X2 — 16 April, 09:48:03
Channel scan for flow cell AFX570 has found a total of 53 pores. 53 pores available for immediate sequencing

X2 — 16 April, 09:46:50
Performing Channel Scan

X1 — 16 April, 09:46:18
Channel scan for flow cell AGE388 has found a total of 55 pores. 55 pores available for immediate sequencing

Hardware check

IMPORTANT

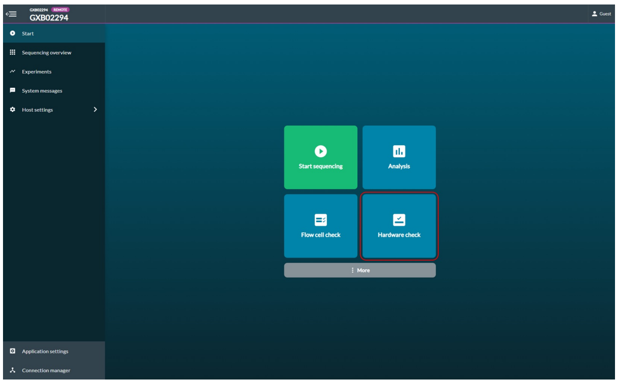
Hardware check

A hardware check must be performed on all new devices or when software has been upgraded. This uses the Configuration Test Cell(s) (CTC), which comes pre-inserted into your device in place of flow cells.

Note: If using a Flongle flow cell, we recommend regularly checking the Flongle adapter by inserting the an **empty** adapter and completing a hardware check. For checking the device or flow cell position, please use a MinION CTC, even if a Flongle flow cell will be used for sequencing.

1 **Insert a Configuration Test Cell (CTC) into the device.**

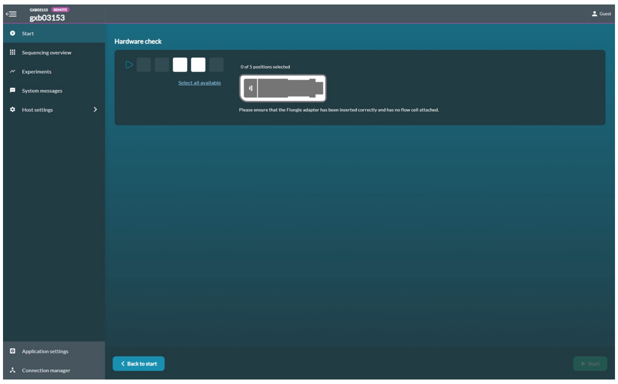
2 **Navigate to the start homepage and select 'Hardware Check' option.**



3 **Select 'Start' for the check to begin.**

Flongle:

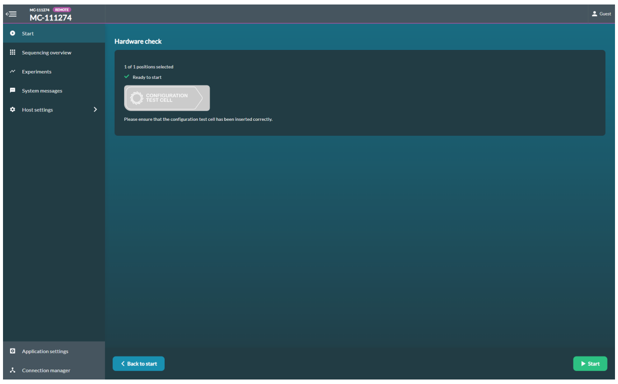
Note: To check the Flongle adapter, insert the EMPTY adapter. To check device or position, insert only a MinION CTC.



0 of 5 positions selected

Please ensure that the Flongle adapter has been inserted correctly and has no flow cell attached.

MinION:

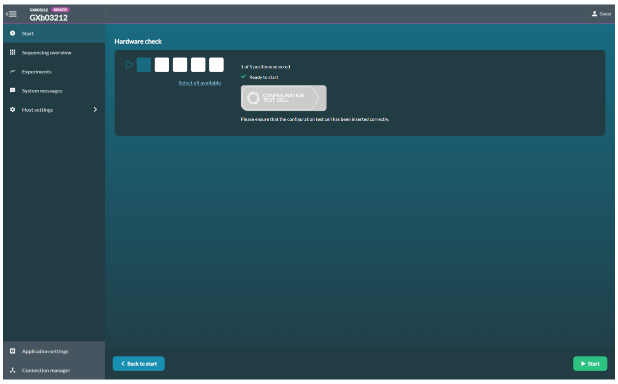


1 of 1 positions selected

✓ Ready to start

Please ensure that the configuration test cell has been inserted correctly.

GridION:

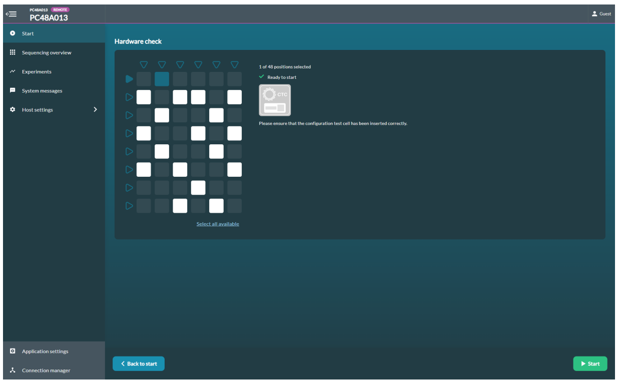


1 of 5 positions selected

✓ Ready to start

Please ensure that the configuration test cell has been inserted correctly.

PromethION:



1 of 48 positions selected

✓ Ready to start

Please ensure that the configuration test cell has been inserted correctly.

You will be automatically navigated to the Sequencing Overview page.

A loading bar will be displayed under the flow cell during the checks.

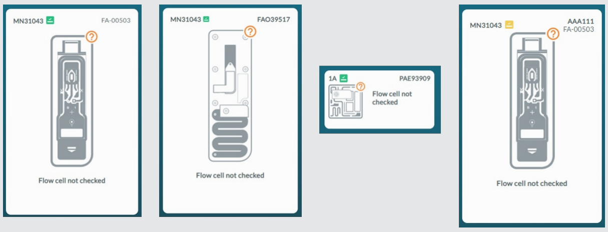
END OF STEP

The hardware check will complete after approximately one minute.

Hardware check **pass** is indicated by a **green check icon**.

A **fail** is indicated by an **orange check icon**.

1. Flongle pass
2. MinION pass
3. PromethION pass
4. Flongle fail



If the hardware check fails, remove and reinsert the CTC, and run a harware check again. If the check fails for a second time, please contact Technical Support via email (support@nanoporetech.com) or via LiveChat in the Nanopore Community.

Flow cell check

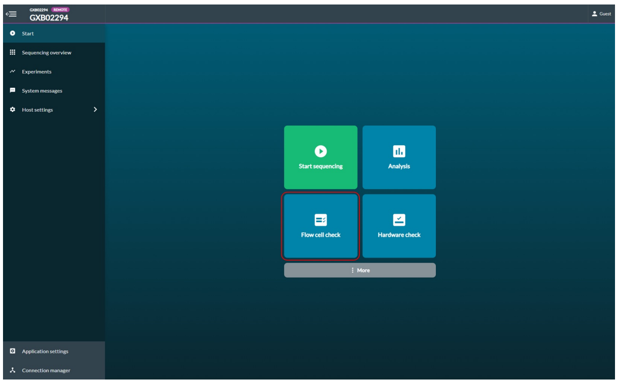
Flow cell check

A flow cell check must be carried out before loading a DNA or RNA library to assess the number of pores available.

Oxford Nanopore Technologies will replace any flow cell that falls below the warranty number of active pores within three months of purchase, provided the result is reported within two days of performing the flow cell check and the storage recommendations have been followed.

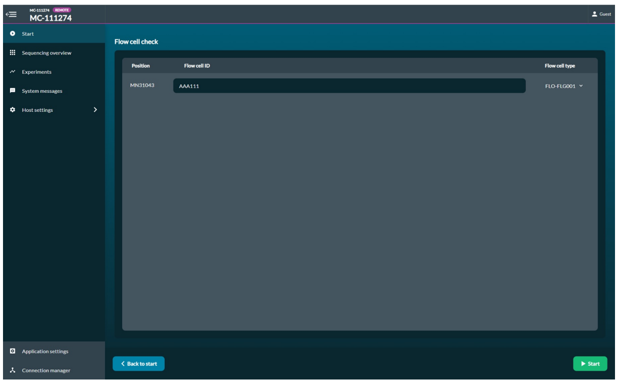
Flow cell	Minimum number of active pores covered by warranty
Flongle Flow Cell (FLO-FLG001)	50
MinION/GridION Flow Cell	800
PromethION Flow Cell	5000

1 Navigate to the start homepage and select 'Flow Cell Check'.



2 Choose the flow cell type from the dropdown menu and select 'Start' to begin.

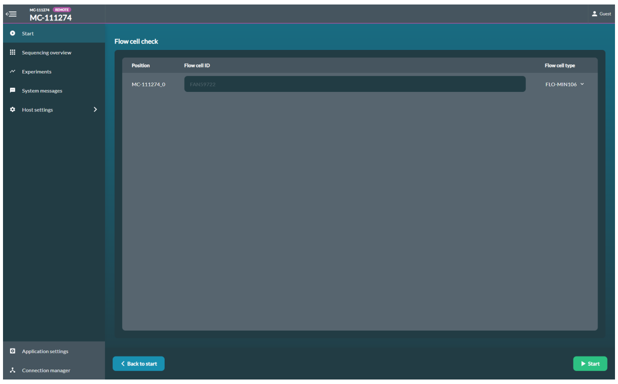
Flongle:
Note: ensure to fill in the flow cell ID for Flongle



Flow cell check

Position	Flow cell ID	Flow cell type
MN31043	AAA111	FLO-FLG001

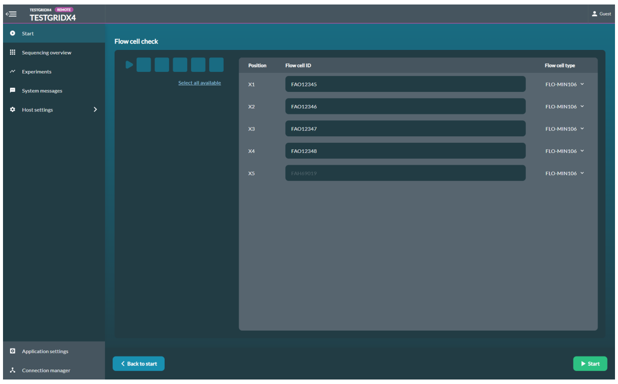
MinION:



Flow cell check

Position	Flow cell ID	Flow cell type
MC-111274_0	FAN59722	FLO-MIN106

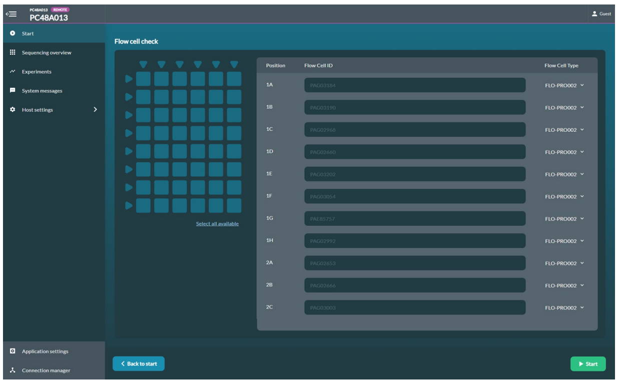
GridION:



Flow cell check

Position	Flow cell ID	Flow cell type
X1	FAO12345	FLO-MIN106
X2	FAO12346	FLO-MIN106
X3	FAO12347	FLO-MIN106
X4	FAO12348	FLO-MIN106
X5	FAH69019	FLO-MIN106

PromethION:



Flow cell check

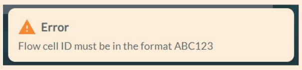
Position	Flow Cell ID	Flow Cell Type
1A	PAG03184	FLO-PRO002
1B	PAG03190	FLO-PRO002
1C	PAG02968	FLO-PRO002
1D	PAG02660	FLO-PRO002
1E	PAG03202	FLO-PRO002
1F	PAG03054	FLO-PRO002
1G	PAE85757	FLO-PRO002
1H	PAG02992	FLO-PRO002
2A	PAG02653	FLO-PRO002
2B	PAG02666	FLO-PRO002
2C	PAG03003	FLO-PRO002

IMPORTANT

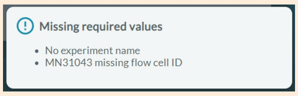
For Flongle, flow cell ID is not automatically assigned in MinKNOW. It is imperative the flow cell ID is entered in the correct format: ABC123 i.e. ([A-Z] x 3 [0-9] x 3).

The ID is case sensitive with no spaces.
The MinKNOW software will not allow you to proceed until the flow cell ID has been entered correctly.

The user will be notified in the bottom right corner of the GUI if any information entered is in the wrong format or missing.

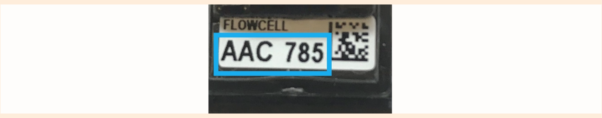


⚠ Error
Flow cell ID must be in the format ABC123



Missing required values
- No experiment name
- MN31043 missing flow cell ID

The Flongle flow cell ID is shown in the blue blox below:



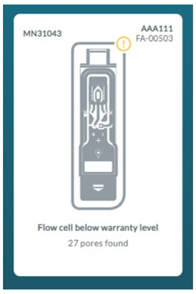
You will be automatically navigated to the Sequencing Overview page.

A loading bar will be displayed under the flow cell during the checks.

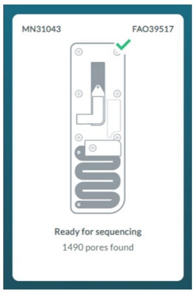
Flow cell health indicators

The quality of the flow cell will be shown as one of the three outcomes on the Sequencing Overview page:

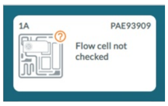
1. **Yellow exclamation mark:** The number of sequencing pores is below warranty.
2. **Green tick:** The number of sequencing pores is above warranty and ready for sequencing.
3. **Question mark:** A flow cell check has not been run on the flow cell during this MinKNOW session.



MN31043 AAA111 FA-00503
Flow cell below warranty level
27 pores found



MN31043 FAO39517
Ready for sequencing
1490 pores found



1A PAE93909
Flow cell not checked

Note: the indicator of quality will only remain visible during the MinKNOW session when testing occured. Once that MinKNOW session has ended, the status of the flow cell will be erased.

Experiments page

Experiments page

The experiments page displays summary information for all sequencing flow cells and device checks carried out on the device.

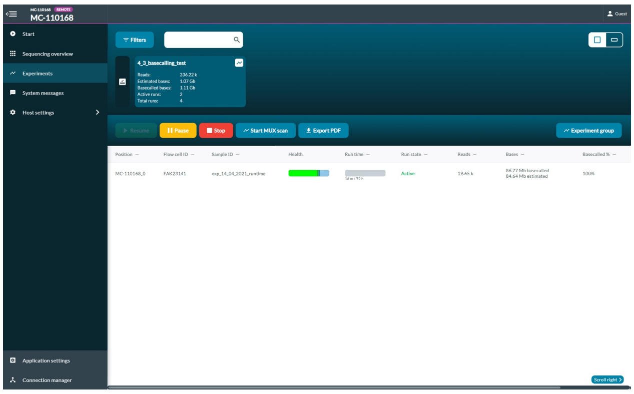
Previous runs can be viewed here until MinKNOW service is restarted (e.g. after a device reboot).

From this page, the user is able to control specific runs and identify real-time information, including flow cell health and reads, giving users real-time feedback for sequencing flow cells.

- **Run statistics:** The total number of reads, estimated and basecalled bases across an experiment, and number or active and total runs
- **Run time:** The duration of the experiment
- **Run state:** The current state of the sequencing run; 'Active', 'Basecalling', 'Complete', 'Stopped with error'
- **Health:** The current flow cell health

The blue panel displays a summary of sequencing experiments and the white panel displays status information of a specific run.

MinION/Flongle:

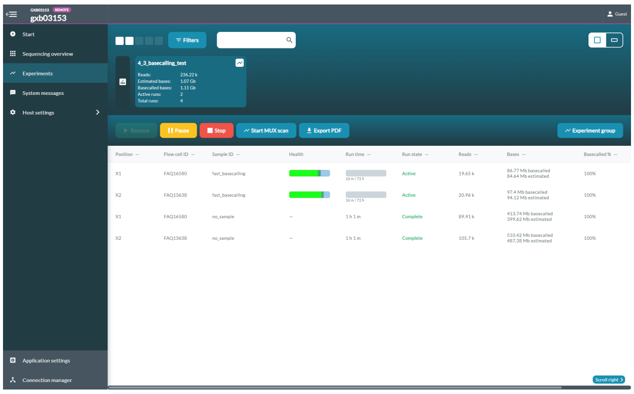


4_3_basecalling_test

Reads:	236.22 k
Estimated bases:	1.07 Gb
Basecalled bases:	1.11 Gb
Active runs:	2
Total runs:	4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
MC-110168_0	FAK23141	exp_14_04_2021_runtime		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%

GridION:

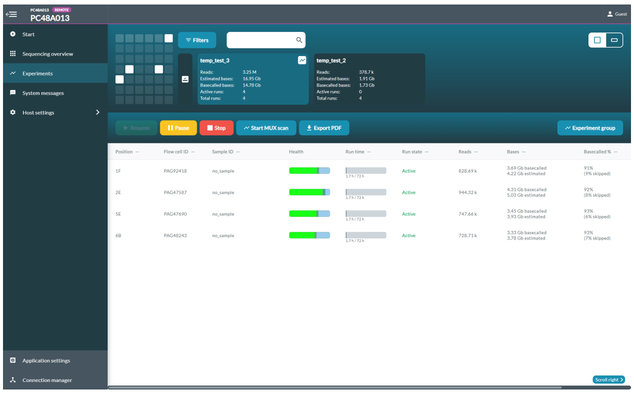


4_3_basecalling_test

Reads:	236.22 k
Estimated bases:	1.07 Gb
Basecalled bases:	1.11 Gb
Active runs:	2
Total runs:	4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%
X2	FAQ13638	fast_basecalling		16 m / 72 h	Active	20.96 k	97.4 Mb basecalled 94.12 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

PromethION:



temp_test_3

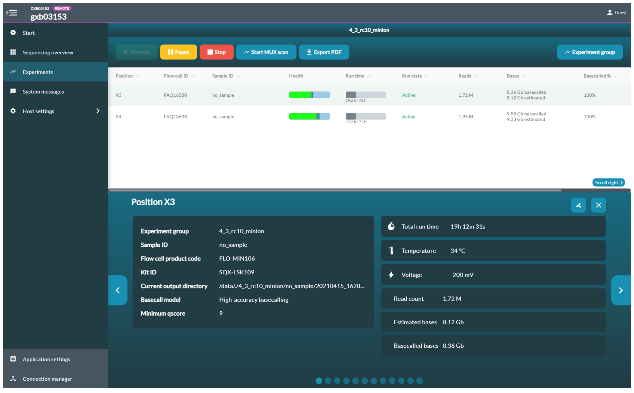
Reads:	3.25 M
Estimated bases:	16.95 Gb
Basecalled bases:	14.78 Gb
Active runs:	4
Total runs:	4

temp_test_2

Reads:	378.7 k
Estimated bases:	1.91 Gb
Basecalled bases:	1.73 Gb
Active runs:	0
Total runs:	4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
1F	PAG92418	no_sample		1.7 h / 72 h	Active	828.69 k	3.69 Gb basecalled 4.22 Gb estimated	91% (9% skipped)
2E	PAG47587	no_sample		1.7 h / 72 h	Active	944.32 k	4.31 Gb basecalled 5.03 Gb estimated	92% (8% skipped)
5E	PAG47690	no_sample		1.7 h / 72 h	Active	747.66 k	3.45 Gb basecalled 3.93 Gb estimated	93% (6% skipped)
6B	PAG48243	no_sample		1.7 h / 72 h	Active	728.71 k	3.33 Gb basecalled 3.78 Gb estimated	93% (7% skipped)

For more status information of a specific run, select the run to open the quick view, including current temperature and voltage.



4_3_rc10_minion

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X3	FAQ16580	no_sample		19.2 h / 72 h	Active	1.72 M	8.36 Gb basecalled 8.12 Gb estimated	100%
X4	FAQ13638	no_sample		19.2 h / 72 h	Active	1.95 M	9.58 Gb basecalled 9.33 Gb estimated	100%

Position X3

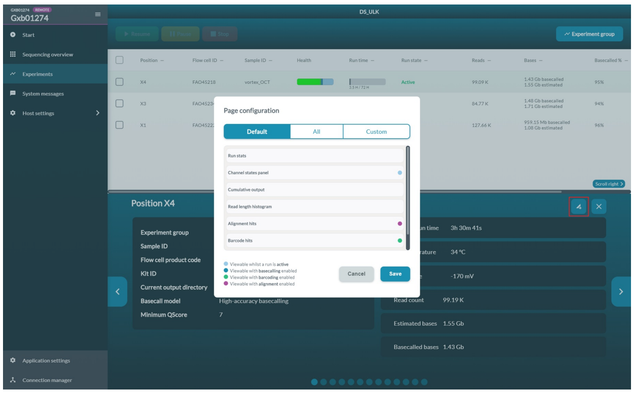
Experiment group	4_3_rc10_minion
Sample ID	no_sample
Flow cell product code	FLO-MIN106
Kit ID	SQK-LSK109
Current output directory	/data/./4_3_rc10_minion/no_sample/20210415_1628...
Basecall model	High-accuracy basecalling
Minimum qscore	9

Total run time	19h 12m 31s
Temperature	34 °C
Voltage	-200 mV
Read count	1.72 M
Estimated bases	8.12 Gb
Basecalled bases	8.36 Gb

Page configuration

Page configuration allows users to choose which graphs to generate in the quick view of an experiment.

To open page configuration, select a run in the white panel to open the quick view and select the highlighted button to choose which graphs to display.



Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X4	FAO45218	vortex_OCT		3.5 H / 72 H	Active	99.09 K	1.43 Gb basecalled 1.55 Gb estimated	95%
X3	FAO4523					84.77 K	1.48 Gb basecalled 1.71 Gb estimated	94%
X1	FAO4522					127.66 K	959.15 Mb basecalled 1.08 Gb estimated	96%

Page configuration

Default | All | Custom

- Run stats
- Channel states panel
- Cumulative output
- Read length histogram
- Alignment hits
- Barcode hits

Viewable whilst a run is **active**
Viewable with **basecalling** enabled
Viewable with **barcoding** enabled
Viewable with **alignment** enabled

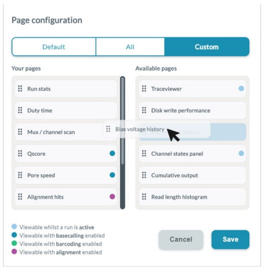
Cancel | Save

Experiment group
Sample ID
Flow cell product code
Kit ID
Current output directory
Basecall model: High-accuracy basecalling
Minimum QScore: 7
Run time: 3h 30m 41s
Temperature: 34 °C
-170 mV
Read count: 99.19 K
Estimated bases: 1.55 Gb
Basecalled bases: 1.43 Gb

Tabs:

- **Default:** Default graphs available
- **All:** All graphs available
- **Custom:** Users can move graphs from 'Available pages' to 'Your pages' to display a custom order of graphs in the GUI. On this page, users can also choose graph order.

To use, **select** the graph and **drag**, then click **Save**.



Page configuration

Default | All | Custom

Your pages	Available pages
Run stats	Traceviewer
Duty time	Disk write performance
Mux / channel scan	Bias voltage history
Qscore	Channel states panel
Pore speed	Cumulative output
Alignment hits	Read length histogram

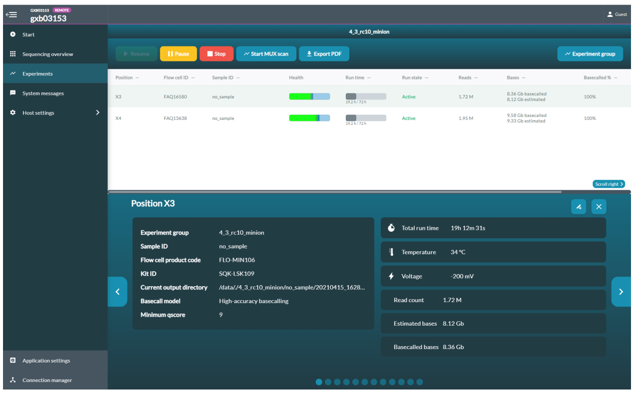
Viewable whilst a run is **active**
Viewable with **basecalling** enabled
Viewable with **barcoding** enabled
Viewable with **alignment** enabled

Cancel | Save

Graphs

Select **Experiment Groups** in the top rigth corner and swipe sideways to view all the graphs presenting data form all previous runs of the same experiment name, including cumulative output for individual and multiple flow cells.

Use the quick view to display information and graphs of specific runs. To open, select a specific run in the white panel and use the arrows to navigate between the graphs



Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X3	FAQ16580	no_sample		19.2 h / 72 h	Active	1.72 M	8.36 Gb basecalled 8.12 Gb estimated	100%
X4	FAQ13638	no_sample		19.2 h / 72 h	Active	1.95 M	9.58 Gb basecalled 9.33 Gb estimated	100%

Experiment group: 4_3_rc10_minion
Sample ID: no_sample
Flow cell product code: FLO-MIN106
Kit ID: SQK-LSK109
Current output directory: /data/./4_3_rc10_minion/no_sample/20210415_1628...
Basecall model: High-accuracy basecalling
Minimum qscore: 9
Total run time: 19h 12m 31s
Temperature: 34 °C
Voltage: -200 mV
Read count: 1.72 M
Estimated bases: 8.12 Gb
Basecalled bases: 8.36 Gb

For more information about the graphs, refer to **Check and monitoring** in the 'Monitoring and troubleshooting your sequencing run' section.

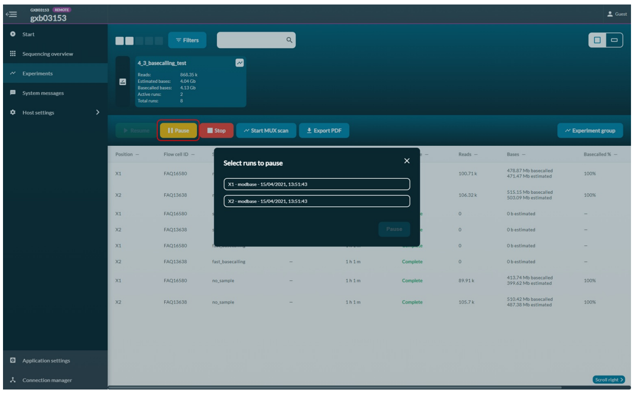
Pausing

For PromethION and GridION, the user is able to pause a single flow cell in a particular position (a run) or every flow cell (an experiment).

For MinION devices, the single flow cell is paused using the same method.

Pausing works by dropping the voltage potential over the membrane to 0 mV to maintain a safe environment to add components, such as fuel, more DNA/RNA library or nucleases for a flow cell wash. Data acquisition will continue during this period, as this prevents sequencing data from being lost.

To pause, click 'Pause' to open a dialogue box and select which flow cell(s) to pause and click 'Pause'.



Select runs to pause

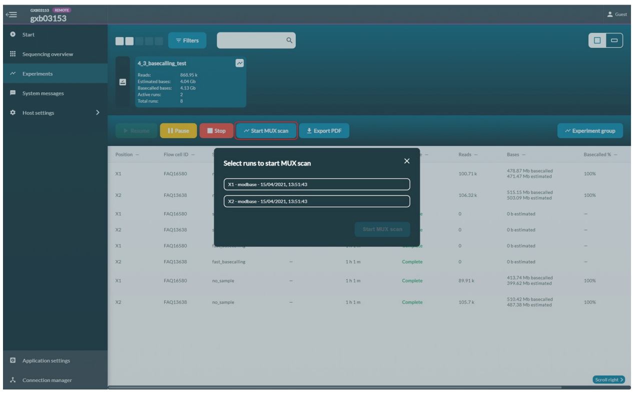
X1 - modbase - 15/04/2021, 13:51:43

X2 - modbase - 15/04/2021, 13:51:43

Triggering a mux scan

The mux scan is used to assess the quality of the four wells in each channel to select the best performing pores. A new mux scan can be triggered every time a sequencing experiment is resumed after a pause (e.g. for a flow cell wash), or if the number of sequencing pores has significantly dropped during an experiment.

Navigate to the **Experiments** page and click **Start MUX scan** and choose which flow cell to scan.



Select runs to start MUX scan

X1 - modbase - 15/04/2021, 13:51:43

X2 - modbase - 15/04/2021, 13:51:43

Sample sheet upload

For GridION and PromethION experiments, or when multiple MinION Mk1Bs or Mk1Cs are running simultaneously, it may be preferred to upload sample names and corresponding flow cell positions from a CSV file, rather than manually.

For this, the information in the CSV file needs to be grouped in one of the following formats:

`flow_cell_id,position_id,sample_id,experiment_id,flow_cell_product_code,kit`

1. `flow_cell_id`: Define the flow cell ID which applies to the sample sheet row.
    - Used to identify which positions to apply values to in MinKNOW
    - This is optional if `position_ID` is specified and the flow cell has the `flow_cell_id` on the EEPROM
    - If `position_id` and `flow_cell_id` are both defined, the `flow_cell_id` will be validated against the EEPROM value
    - `flow_cell_id` will be set against the `user_specified_flow_cell_id` when starting a protocol
2. `position_id`: Define the flow cell position which applies to the sample sheet row.
    - Used to identify which position to apply values to in MinKNOW
    - This is optional if `flow_cell_id` is specified.
3. `sample_id`: Define the sample ID to be applied in the run
    - This is optional
    - An individual position can only have one `sample_id` assigned to it when starting a run
4. `experiment_id`: Define the experiment ID to be applied
    - Each row in the sample sheet must contain the same `experiment_id` value for the sample sheet to only have a single `experiment_id` value defined overall. All entries on each row will be validated.
5. `flow_cell_product_code`: Define the product code of the flow cell
    - Used to find a protocol to start
    - This is required if the product code is not available on the EEPROM.
6. `kit`: Define the kit and any expansion kits used with the current sample

   - Used to find a protocol to start
   - The sample sheet must contain only 1 sequencing kit but expansion kits are not limited
   - If expansion kits are additionally defined, they should be separated by a space: e.g. `SQK-LSK109 EXP-NBD104 EXP-NBD114` For experiments that involve barcoding, an alias is associated with each barcode (or pair of barcodes where appropriate to the kit being utilised). Additional columns are available for barcoding runs:

7. `alias`: User specified string which applies a given label to a specific barcode or barcode pair
   - e.g. `patient_id_5`
8. `type`: One of the options: `test_sample` , `positive_control` , `negative_control` , `no_template_control`
   - This is optional

One or two columns define the barcode or barcode pair. The exact form is dependent on whether single or dual barcoding is used:

1. Single barcoding: The sample sheet contains one barcoding arrangement column:
   - `barcode`: The barcode identifier for the row e.g. `barcode01`
2. Dual barcoding: The sample sheet contains two barcoding arrangement columns:
   - `internal_barcoding`: The internal barcode identifier for the row e.g. `internal01`
   - `external_barcode`: The external barcode identifier for the row e.g. `external01` Column titles are defined within the first row of the sample sheet. These must be defined in lowercase using the above mentioned column title values.

Sample sheet validation occurs against the hardware and between rows in the sample sheet to ensure validity. Validation occurs when the sample sheet is loaded. Validation and importing the sample sheet requires all the flow cells are inserted into their positions.

Sample sheet examples:

Single barcode

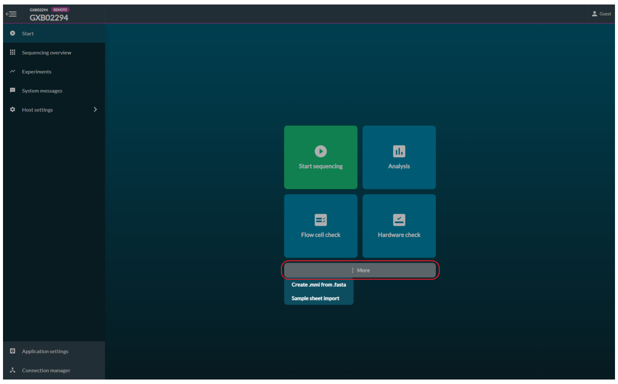
```
flow_cell_id,kit,sample_id,experiment_id,barcode,alias,type
FA026858,SQK-RBK004,barcoding_run,sequencing_20200522,barcode01,patient_id_5,test_sample
FA026858,SQK-RBK004,barcoding_run,sequencing_20200522,barcode02,patient_id_6,test_sample
```

Dual barcode

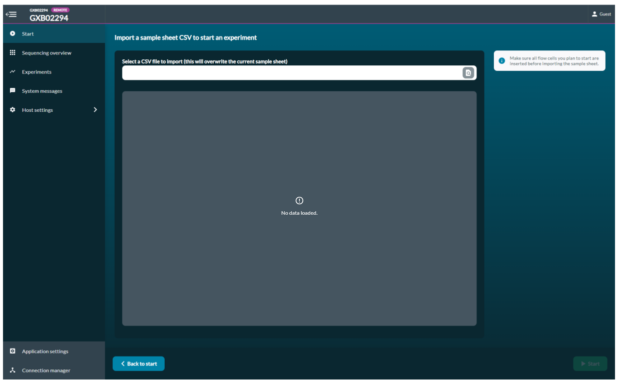
```
position_id,kit,sample_id,experiment_id,external_barcode,internal_barcode,alias,type
X1,SQK-LSK109 EXP-NBD104 XP-NBD114,dual_barcoding_run,barcoding_20200522,external01,internal01,patient_id_5,test_sample
X1,SQK-LSK109 EXP-NBD104 XP-NBD114,dual_barcoding_run,barcoding_20200522,external01,internal02,patient_id_6,test_sample
```

1 Navigate to the Start page

2 Select 'More' and click 'Sample sheet import'.



3 Select and upload the CSV file.



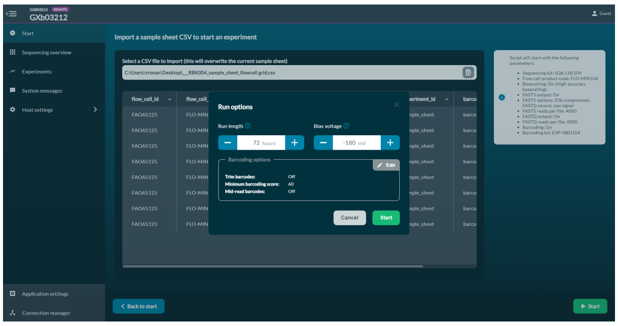
Import a sample sheet CSV to start an experiment

Select a CSV file to import (this will overwrite the current sample sheet)

Make sure all flow cells you plan to start are inserted before importing the sample sheet.

No data loaded.

4 **Click 'Start' when the CSV file has been uploaded. A dialogue box will open to select the parameters for the sequencing run.**



Import a sample sheet CSV to start an experiment

Select a CSV file to import (this will overwrite the current sample sheet)

C:\Users\rronan\Desktop__RBK004_sample_sheet_flowcell grid.csv

Run options

Run length: 72 hours

Bias voltage: -180 mV

Barcoding options

Trim barcodes: Off
Minimum barcoding score: 60
Mid-read barcodes: Off

Script will start with the following parameters:

- Sequencing kit: SQK-LSK109
- Flow cell product code: FLO-MIN106
- Basecalling: On (High-accuracy basecalling)
- FAST5 output: On
- FAST5 options: Zlib compression, FASTQ record, raw signal
- FAST5 reads per file: 4000
- FASTQ output: On
- FASTQ reads per file: 4000
- Barcoding: On
- Barcoding kit: EXP-NBD104

Sample sheet errors:

- Position defined using position_id does not exist
- Position defined using flow_cell_id cannot be found
- flow_cell_id and position_id are used but the flow_cell_id does not match that on the EEPROM (if there is a value on the EEPROM)
- flow_cell_product_code is used but the value doesn't match the EEPROM value (if there is a value on the EEPROM)
- Different sample_id values are assigned against the same position whilst setting up a run
- flow_cell_product_code and kit do not match
- Zero or more than one sequencing kit listed in kit
- Barcode kits in kit do not exist as an option on the selected protocol
- type must be a valid selection from the list of valid types outlined above
- experiment_id must exist within each row and must all have the same value

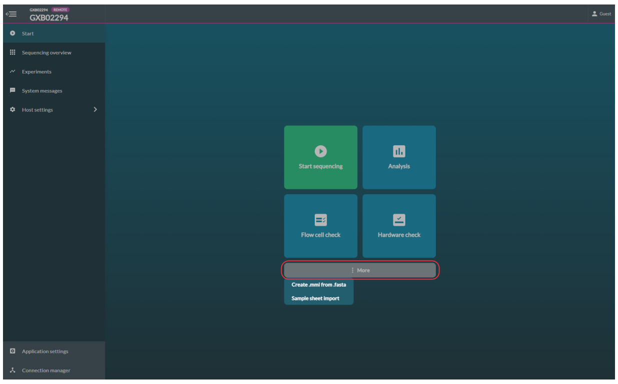
MiniMap index generation

Generating a MiniMap index file prior to starting a run

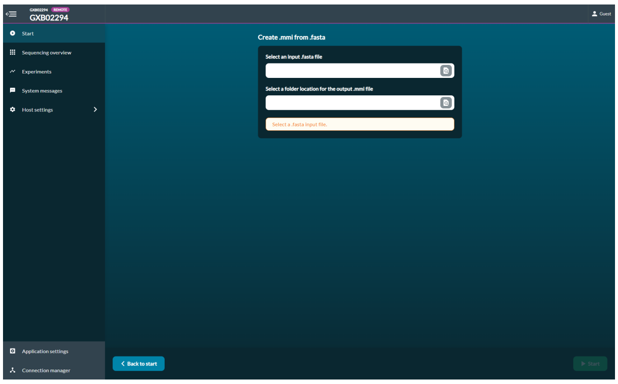
To save time when setting up a sequencing run with live alignment, a MiniMap index file can be created from a FASTA file from the MinKNOW Start page, prior to set up.

1 **Navigate to the Start page**

2 **Select 'More' and click 'Create .mmi from .fasta'.**



3 **Select an input .fasta file to upload and a folder location for the output .mmi file.**

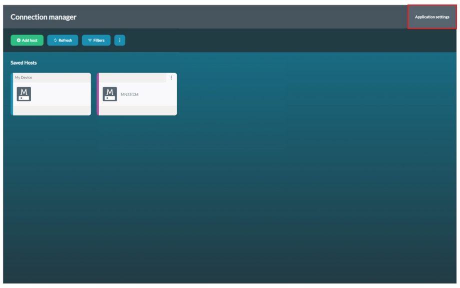


Select a .fasta input file.

Application settings

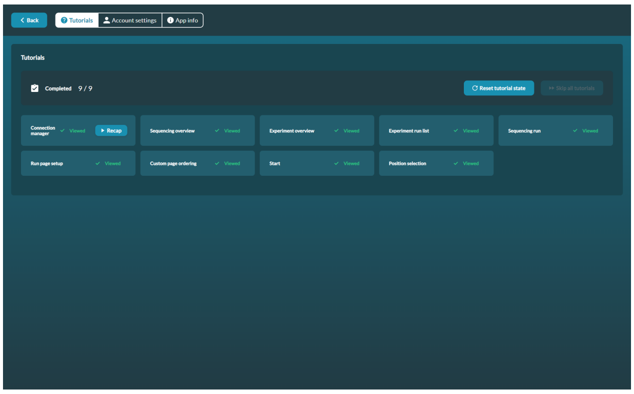
Application settings contains the tutorials, account/login settings and MinKNOW GUI information.

Navigate to application settings from the sidebar or Connection Manager.



Tutorials

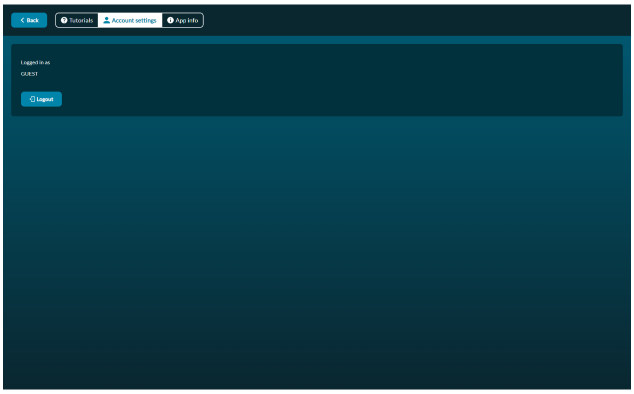
Tutorials are available here. Users can use **Recap** to view a specific tutorial video or use **Reset tutorial state** to go through all the tutorials again.



Account settings

Navigate here to logout from MinKNOW.

User email address used for login is displayed here, unless logged in as a guest.



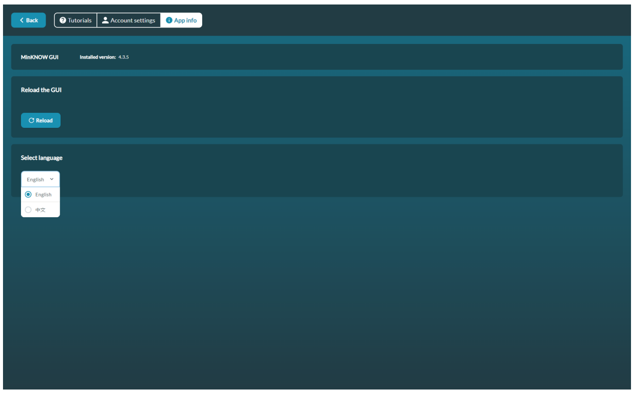
Logged in as
GUEST

App information

The installed version of the MinKNOW GUI is available here.

Click **Reload** to refresh the GUI.

Use **Select language** to change between English and Mandarin.



MinKNOW GUI Installed version: 4.3.5

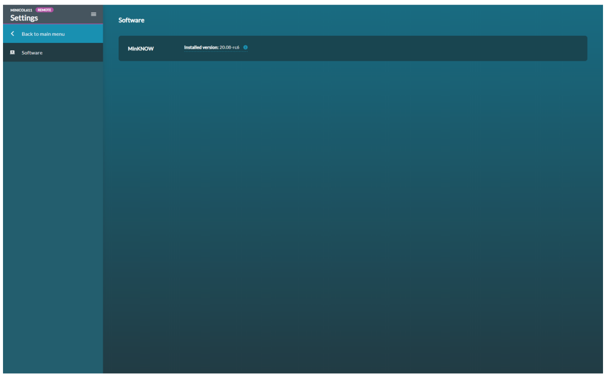
Select language: English

Host settings

Host Settings

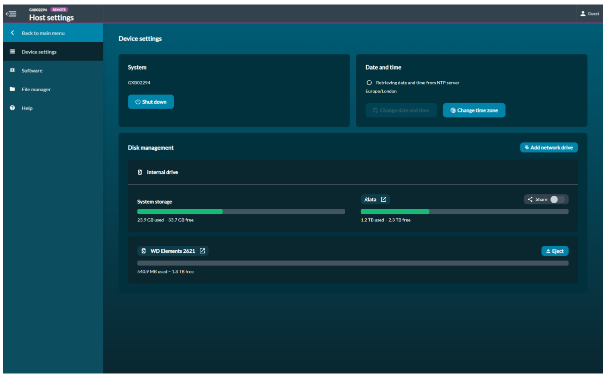
This tab opens the admin interface where users can change device settings.

- Flongle/MinION:



MinKNOW Installed version: 20.08-rc6

- GridION/PromethION:



Retrieving date and time from NTP server

Europe/London

23.9 GB used - 33.7 GB free

1.2 TB used - 2.3 TB free

540.9 MB used - 1.8 TB free

Overview

Features of the Host settings

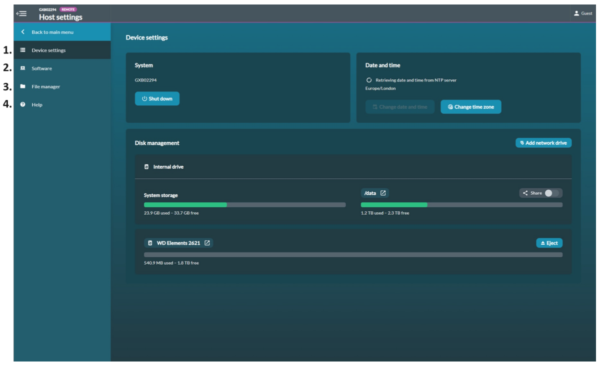
The Host settings have several features to co-ordinate the device. From this area, users are provided with information on system parameters, such as storage, date/time and IP address.

Note: Host settings differ between devices as explained later in this protocol.

1. Device Settings: The devices can be shutdown and rebooted from this page and settings for the time and date can be altered. Disk space can be managed and peripherals may also be added and ejected from here.
2. Software: Users are able to download the latest software updates from this page. The option to update will only appear when an update is available.
3. File Manager: Stored data on the device can be managed and transferred.
4. Help: Device logs can be exported using the Export Logs function. Logs and temporary data can also be cleared by using the Clean up function. If troubleshooting of the device is required, a Repair problems function is available as well as a MinKNOW restart option.
5. Network settings: Ethernet and WiFi settings are available here with the option to hotspot the device to connect to a larger screen.

Select **Back to Main Menu** to leave host settings.

- GridION/PromethION:



Retrieving date and time from NTP server

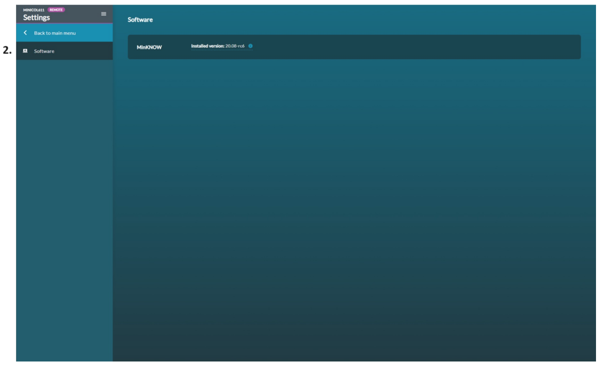
Europe/London

23.9 GB used - 33.7 GB free

1.2 TB used - 2.3 TB free

540.9 MB used - 1.8 TB free

- MinION Mk1B:

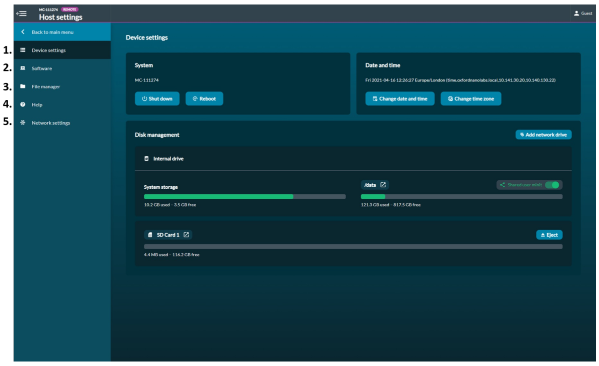


Installed version: 20.08-rc6

- MinION Mk1C

Host settings

Version: MKE_1013_v1_revBZ_11Apr2016



MC-111274

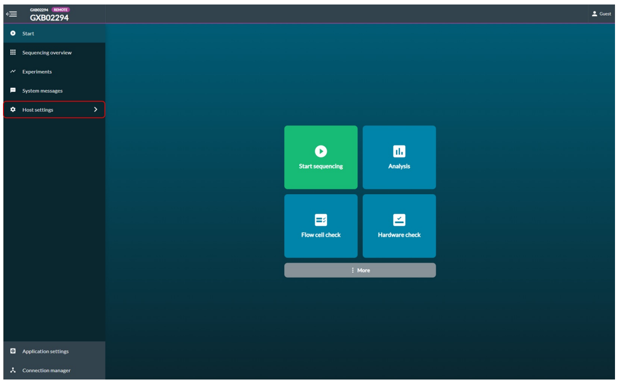
Fri 2021-04-16 12:26:27 Europe/London (time.oxfordnanolabs.local,10.141.30.20,10.140.130.22)

10.2 GB used - 3.5 GB free

121.3 GB used - 817.5 GB free

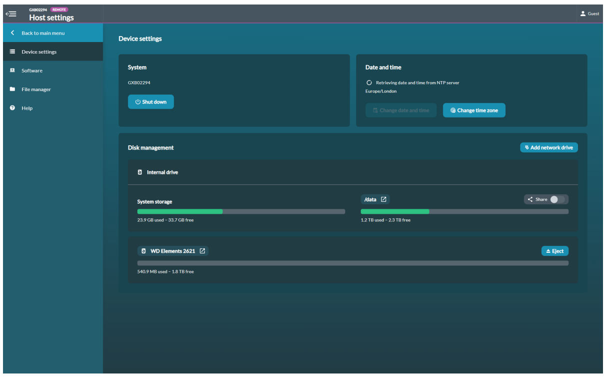
4.4 MB used - 116.2 GB free

1 **Select 'Host Settings' in the side panel to open the options.**



Use the side panel to navigate through the options.

- Device settings:
  Unavailable on MinION Mk1B.



GXB02294

Retrieving date and time from NTP server

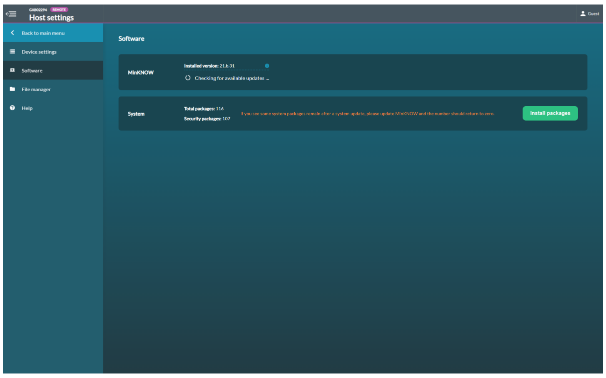
Europe/London

23.9 GB used - 33.7 GB free

1.2 TB used - 2.3 TB free

540.9 MB used - 1.8 TB free

- Software:
  Available on all devices.



Installed version: 21.b.31

Checking for available updates ...

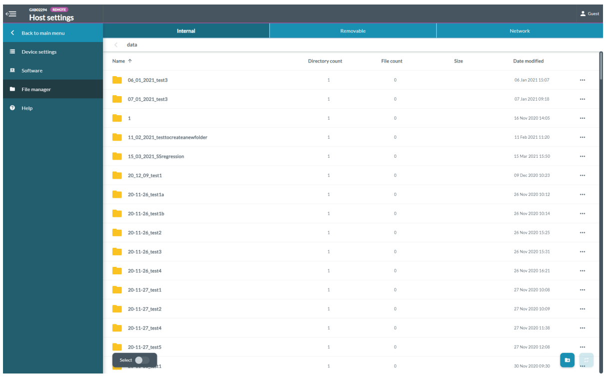
Total packages: 116

Security packages: 107

If you see some system packages remain after a system update, please update MinKNOW and the number should return to zero.

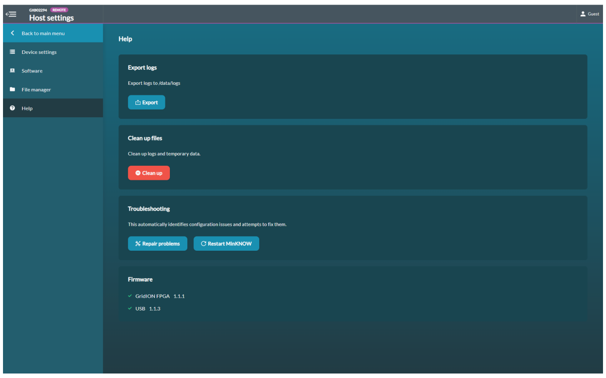
- File manager:

Unavailable on MinION Mk1B.



Name	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression	1	0		15 Mar 2021 15:50
20_12_09_test1	1	0		09 Dec 2020 10:23
20-11-26_test1a	1	0		26 Nov 2020 10:12
20-11-26_test1b	1	0		26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

- Help:
  Available on all devices.



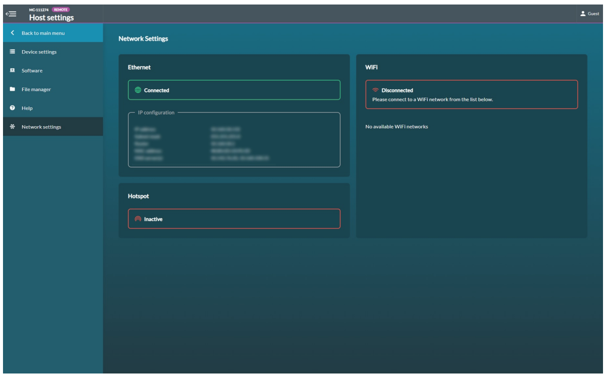
Export logs to /data/logs

Clean up logs and temporary data.

This automatically identifies configuration issues and attempts to fix them.

✓ GridION FPGA 1.1.1
✓ USB 1.1.3

- Network settings:
  Available on MinION Mk1C and MinIT.



Connected

[redacted]

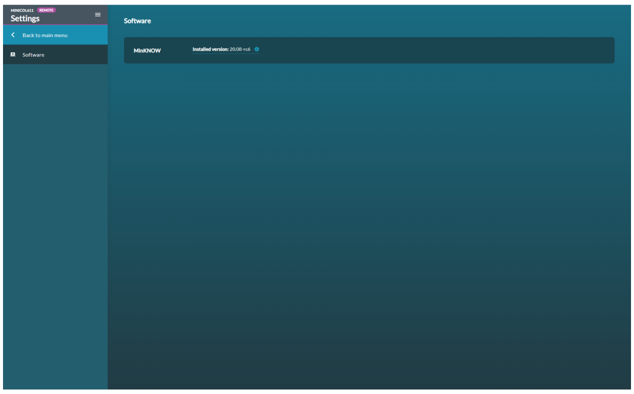
Inactive

Disconnected
Please connect to a WiFi network from the list below.

No available WiFi networks

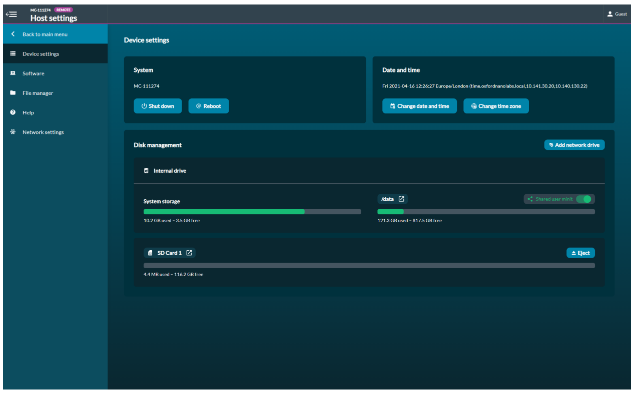
Host settings on different devices:

MinION Mk1B:

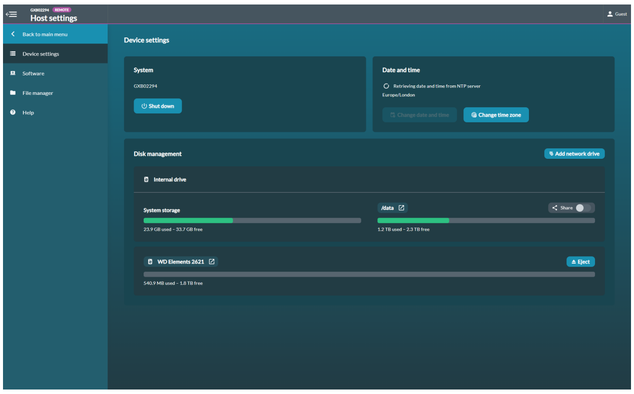


Installed version: 20.08-rc6

MinIN Mk1C:



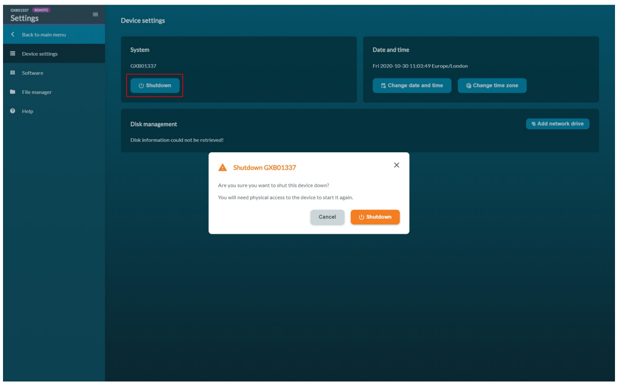
GridION and PromethION:



Shutting down

1 **Click 'Shutdown' in Device Settings.**

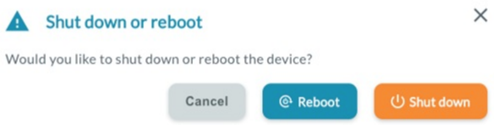
2 **Wait for the Shutdown window to load and click 'Shutdown'.**



Are you sure you want to shut this device down?

You will need physical access to the device to start it again.

Optional Action
For MinION Mk1C, the device can be shutdown by clicking the power button and selecting 'Shutdown'.

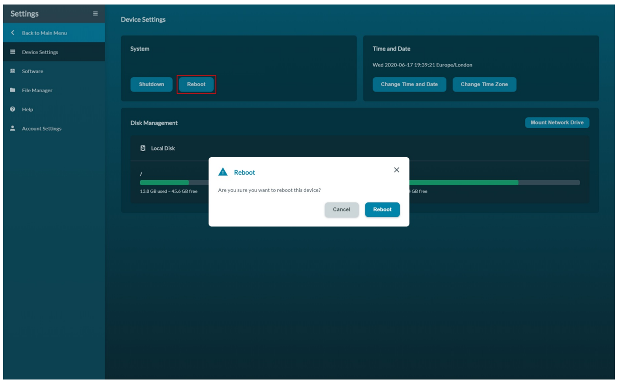


Would you like to shut down or reboot the device?

Rebooting

1 **Select 'Reboot' in Device Settings.**

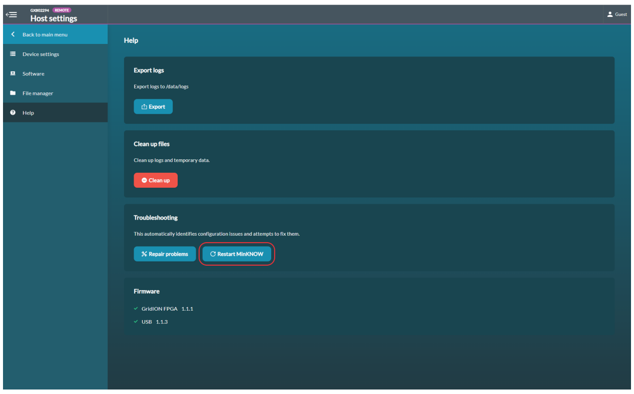
2 **Wait for the Reboot window to load and click 'Reboot'.**



Are you sure you want to reboot this device?

Note: Unavailable on GridION.

To restart MinKNOW on GridION, navigate to 'Help' in the host settings and select 'Restart MinKNOW'.



Export logs to /data/logs

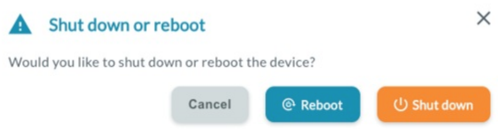
Clean up logs and temporary data.

This automatically identifies configuration issues and attempts to fix them.

GridION FPGA 1.1.1

USB 1.1.3

Optional Action
For MinION Mk1C, the device can be restarted by clicking the power button and selecting 'reboot'.



Would you like to shut down or reboot the device?

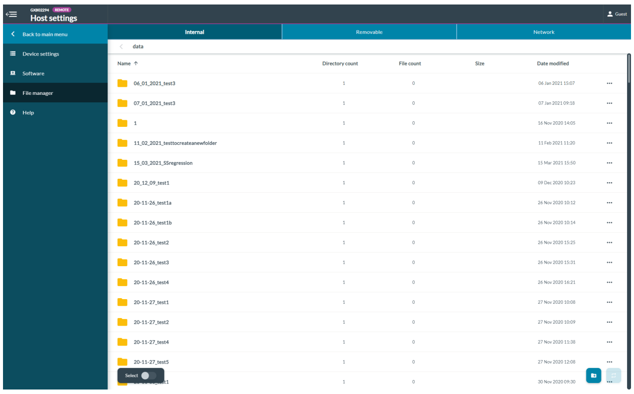
Data management

File manager

Data can be managed and transferred from the file manager tab on the host settings.

Navigate through the tabs to view the data stored:

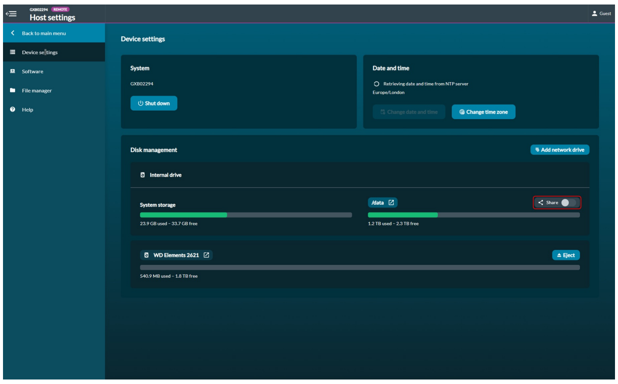
- **Internal** tab: Data stored on the connected sequencing device (MinION Mk1C, GridION, PromethION)
- **Removable** tab: Data stored on a connected removable storage device e.g. USB drive
- **Network** tab: Data stored on a connected network drive. The network drive must be mounted prior.



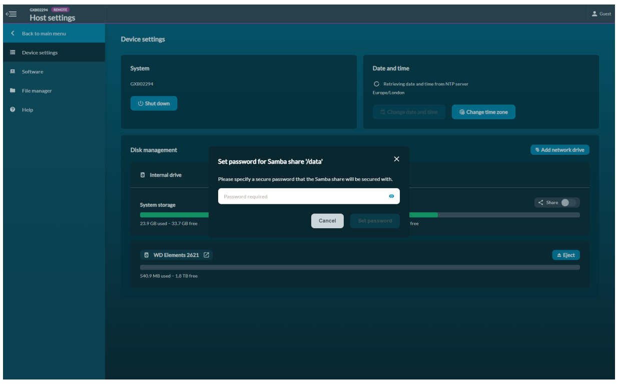
Name	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression	1	0		15 Mar 2021 15:50
20_12_09_test1	1	0		09 Dec 2020 10:23
20-11-26_test1a	1	0		26 Nov 2020 10:12
20-11-26_test1b	1	0		26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

To remotely access the device as a shared/network drive:

1 **Navigate to device settings and switch the 'Share' toggle on.**



2 **A new window will open to create a password for security.**



Set password for Samba share '/data'

Please specify a secure password that the Samba share will be secured with.

3 **Select 'Set password', and after a few seconds the share toggle will be active.**

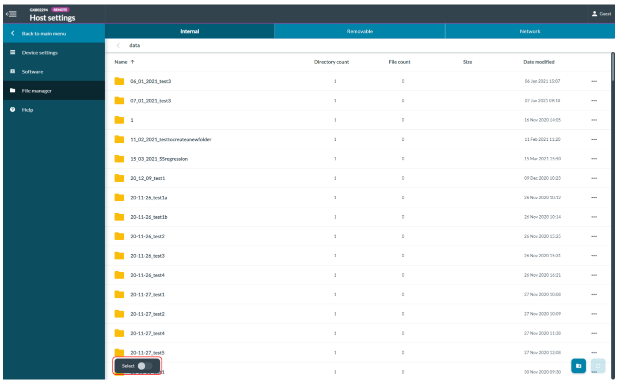
Data will now be able to be shared and accessible on different networks.

Note: The image above is an example of sharing on a GridION. On MinION devices, 'minit' will appear and PromethION devices will show 'prom' when active.

> **TIP**
>
> **To unshare, select the toggle to turn off.**
>
> **Note:** The password will be removed and must be reset when sharing is turned back on.

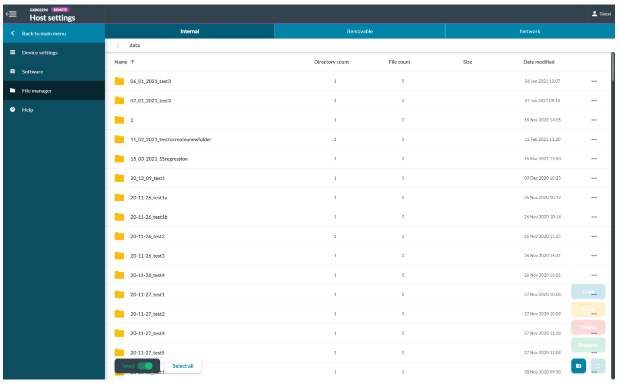
To manage data between the internal, removable and network tabs:

4 **Switch the 'Select' toggle on to select a folder.**



Name ↑	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression	1	0		15 Mar 2021 15:50
20_12_09_test1	1	0		09 Dec 2020 10:23
20-11-26_test1a	1	0		26 Nov 2020 10:12
20-11-26_test1b	1	0		26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

5 **Use the select toggle to open options; 'Select all', 'Copy', 'Move', and 'Delete'.**

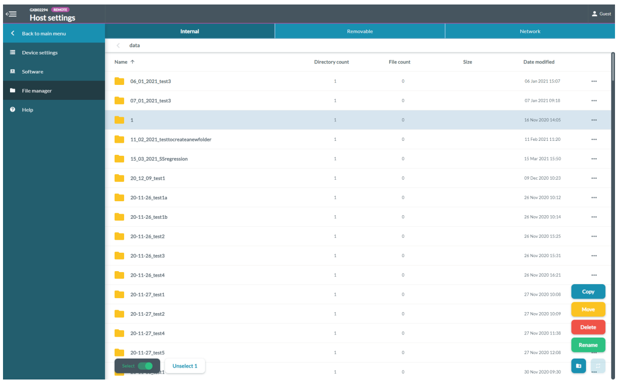


Name ↑	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression	1	0		15 Mar 2021 15:50
20_12_09_test1	1	0		09 Dec 2020 10:23
20-11-26_test1a	1	0		26 Nov 2020 10:12
20-11-26_test1b	1	0		26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

> **TIP**
>
> **To use 'Move' and 'Delete', select a file and choose an option to either move or delete.**

6 **Select the file to copy and click 'Copy'.**

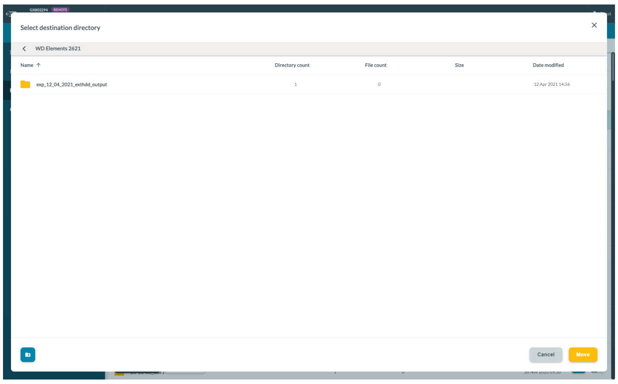
A file or folder can be copied to one of your drives on the computer connected for further analysis. Relevant file output folders labelled .fast5 and .fastq will contain basecalled data.



Name ↑	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression	1	0		15 Mar 2021 15:50
20_12_09_test1	1	0		09 Dec 2020 10:23
20-11-26_test1a	1	0		26 Nov 2020 10:12
20-11-26_test1b	1	0		26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

7 **When you click either 'Copy' or 'Move', a new dialog box will open with a list of drives accessible to the device. Choose a drive to copy or move the data.**

8 **Choose which drive to open to copy or move data.**



Name ↑	Directory count	File count	Size	Date modified
exp_12_04_2021_exthdd_output	1	0		12 Apr 2021 14:56

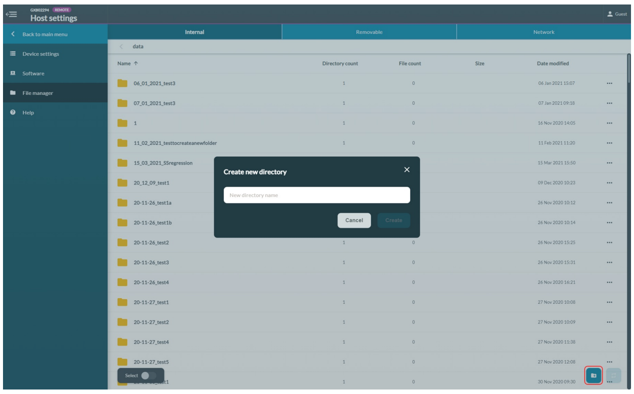
9 **Navigate through the file directory to choose destination and select copy to paste the data in your chosen destination.**

Compatible disk formats:

SD and USB on MinION Mk1C supports DOS FAT, Windows 95 FAT, xFAT, and Linux ext(2|3|4).

New directories can be created from the device GUI using the 'New Directory' option.

Click the icon in the bottom right corner to open Create New Directory and type in a directory name.
Select **Create** and use the refresh button at the top right corner of the GUI for the new directory to appear.



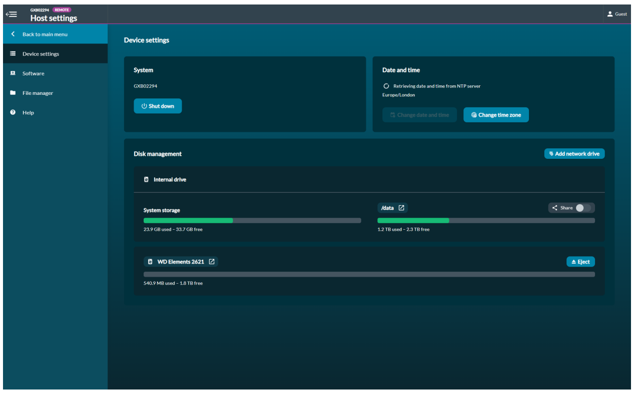
Name ↑	Directory count	File count	Size	Date modified
06_01_2021_test3	1	0		06 Jan 2021 15:07
07_01_2021_test3	1	0		07 Jan 2021 09:18
1	1	0		16 Nov 2020 14:05
11_02_2021_testtocreateanewfolder	1	0		11 Feb 2021 11:20
15_03_2021_SSregression				15 Mar 2021 15:50
20_12_09_test1				09 Dec 2020 10:23
20-11-26_test1a				26 Nov 2020 10:12
20-11-26_test1b				26 Nov 2020 10:14
20-11-26_test2	1	0		26 Nov 2020 15:25
20-11-26_test3	1	0		26 Nov 2020 15:31
20-11-26_test4	1	0		26 Nov 2020 16:21
20-11-27_test1	1	0		27 Nov 2020 10:08
20-11-27_test2	1	0		27 Nov 2020 10:09
20-11-27_test4	1	0		27 Nov 2020 11:38
20-11-27_test5	1	0		27 Nov 2020 12:08
[illegible]	1	0		30 Nov 2020 09:30

Disk management

Navigate to **Device Settings** to view available disk space on the device.
Any peripherals plugged in, including USB, can be ejected using the **Eject** option.

A network drive can also be connected to the device by selecting **Add Network Drive** and filling in the required credentials for connecting using a Samba (SMB) server or a Network File System (NFS).

Please refer to the 'Mount Network Drive' of this protocol for more information.

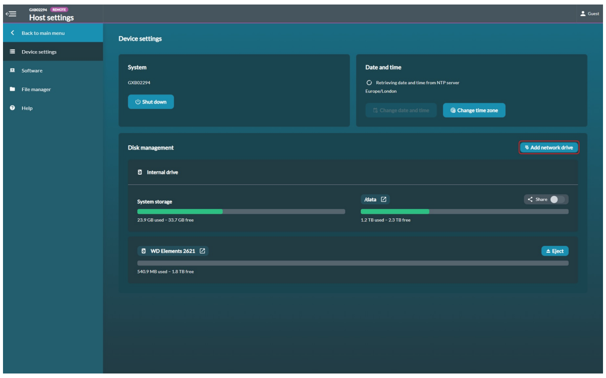


Mount Network Drive

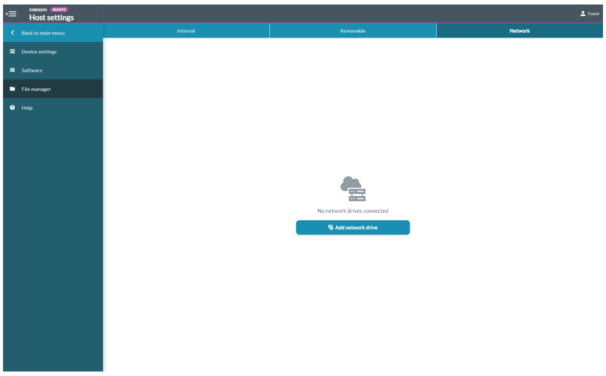
Network drives

Network drives for data storage can be mounted from either the Device Settings or the File Manager in the Network tab:

- Device Settings:



- File Manager:

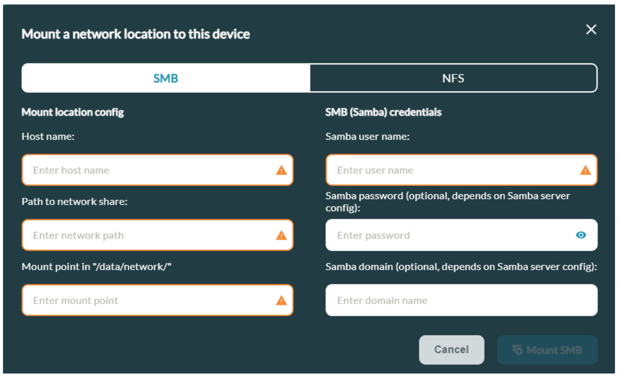


1 **Select 'Mount Network Drive'.**

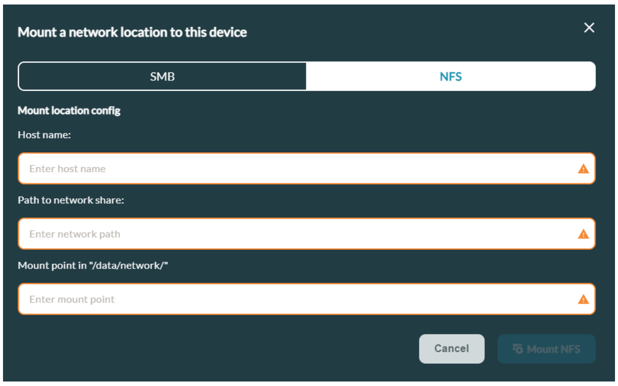
2 **A modal will open. Select either SMB or NFS shares.**

Use SMB for connecting to a shared drive on a Windows server, and NFS for Linux.
If your shared drive is on a macOS machine or NAS (Network-Attached Storage), you can use either SMB or NFS.

SMB:

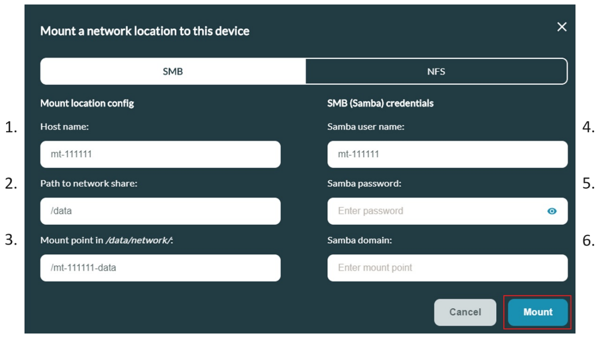


NFS:



3 **To mount SMB shares:**

1. Enter the host name or IP address of the SMB share. This is acquired from your network admin.
2. Enter the drive on the network host that the user wants to share. Take note to start with a '/'. E.g. `/data`.
3. The 'Mount Point' field will auto-populate with the host name and path to the network share. However, the customer is allowed to customise this. In the file manager, it will appear as: `/data/network/mt-111111-data` for example.
4. Add a user name.
5. *(Optional)* Enter a password.
6. *(Optional)* Enter a domain name.
7. Select **Mount**.



Host name: mt-111111

Path to network share: /data

Mount point in */data/network/*: /mt-111111-data

Samba user name: mt-111111

Samba password:

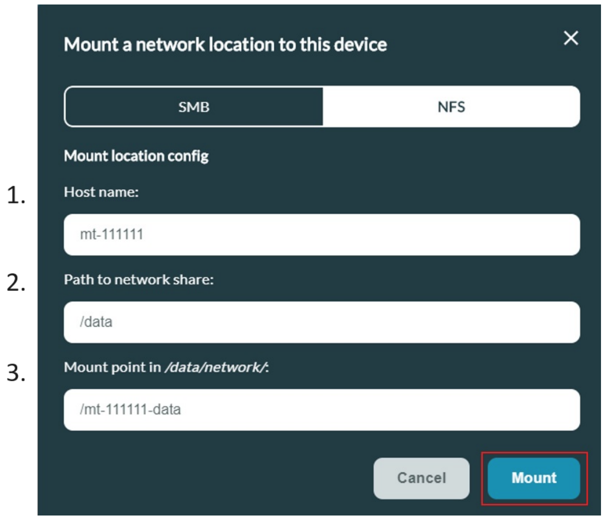
Samba domain:

Providing the settings were correct, the mounted drive will be added to the 'Disk Management' overview. The user will be able to:

- View the space information for the drive
- Unmount the network drive
- A link to jump to the mount point inside the file manager

4 **To mount NFS shares:**

1. Enter the host name or IP address of the NFS share. Obtain this from your network admin.
2. Enter the drive on the network host that the user wants to share. Take note to start with a '/'. E.g. `/data`.
3. The 'Mount Point' field will auto-populate with the host name and path to the network share. However, the customer is allowed to customise this. In the file manager, it will appear as: `/data/network/mt-111111-data` for example.
4. Select **Mount**.



Mount a network location to this device

Mount location config

1. Host name: mt-111111

2. Path to network share: /data

3. Mount point in */data/network/*: /mt-111111-data

Providing the settings were correct, the mounted drive will be added to the 'Disk Management' overview. The user will be able to:

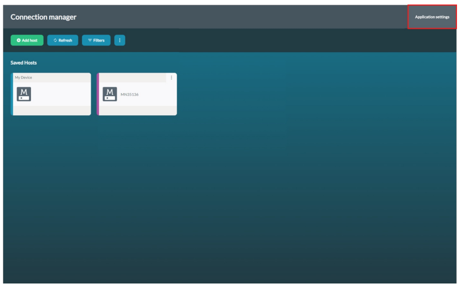
- View the space information for the drive
- Unmount the network drive
- Access a link to jump to the mount point inside the file manager

Tutorials

Tutorials

Tutorials are available on Application Settings to help get users get started and running their experiments as easily as possible.

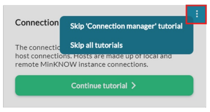
On opening the MinKNOW software, the tutorials will start to navigate the user through the user interface.



Skip tutorials

Tutorials bay be skipped by users familiar with the user interface.

To skip tutorials, select the three dots and choose to either skip all tutorials or just a section.

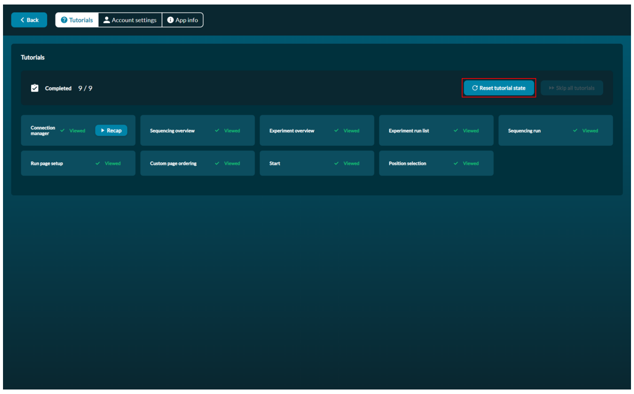


Connection

The connectio
host connections. Hosts are made up of local and remote MinKNOW instance connections.

Review tutorials

Tutorials can be reviewed again by selecting **Reset tutorial state** in the Tutorials panel of Application Settings.



Completed 9 / 9

Connection manager	✓ Viewed
Sequencing overview	✓ Viewed
Experiment overview	✓ Viewed
Experiment run list	✓ Viewed
Sequencing run	✓ Viewed
Run page setup	✓ Viewed
Custom page ordering	✓ Viewed
Start	✓ Viewed
Position selection	✓ Viewed

Network settings and connection

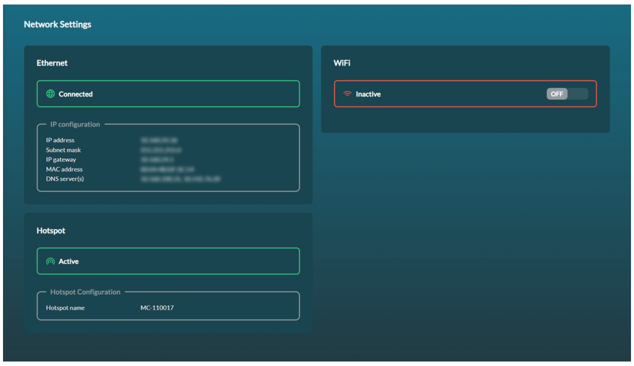
Network settings display internet connectivity information. The MinION Mk1C and MinIT devices can be connected via Ethernet or Wi-Fi.

Both options are supported, however if Ethernet is available, we recommend using Ethernet because the connection will likely be quicker and more reliable.

Ethernet

To connect using Ethernet:

1. Connect a Ethernet cable to the device
2. Navigate to network settings in host settings to confirm connection. IP configuration information will appear when connected.



Ethernet: Connected

IP configuration
IP address [redacted]
Subnet mask [redacted]
IP gateway [redacted]
MAC address [redacted]
DNS server(s) [redacted]

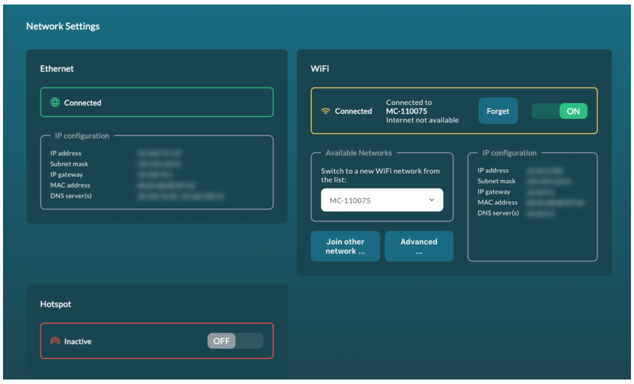
WiFi: Inactive

Hotspot: Active

Hotspot Configuration
Hotspot name MC-110017

Wi-Fi

To connect using Wi-Fi, navigate to network settings and toggle Wi-Fi on. Select a network from the **Available Networks** panel to connect to the internet.



Ethernet: Connected

IP configuration
IP address [redacted]
Subnet mask [redacted]
IP gateway [redacted]
MAC address [redacted]
DNS server(s) [redacted]

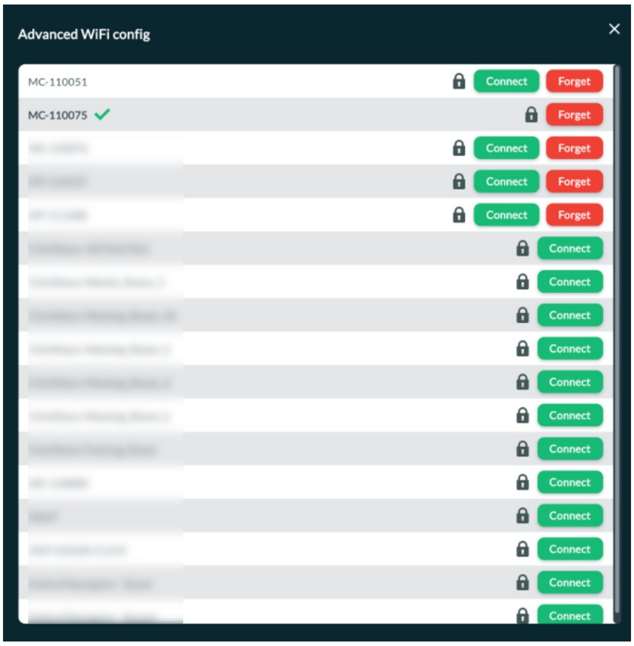
WiFi: Connected — Connected to MC-110075 Internet not available

Switch to a new WiFi network from the list: MC-110075

IP configuration
IP address [redacted]
Subnet mask [redacted]
IP gateway [redacted]
MAC address [redacted]
DNS server(s) [redacted]

Hotspot: Inactive

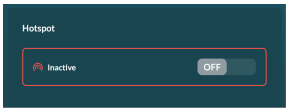
Select **Advanced** to open Advanced Wi-Fi Configurations. From here, all available networks can be viewed, connected or removed.

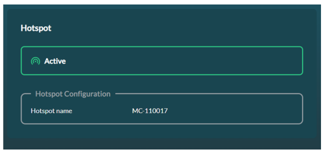


> **IMPORTANT**
>
> **Please note that Wi-Fi connections with captive portals (a web page that the user is required to view and enter login details before access is granted) cannot be used with the MinION Mk1C.**

Hotspot can be activated to allow users to connect to a larger screen.

This can be used to connect to the MinION Mk1C in places where there is no network infrastructure, e.g. in the field. To enable hotspot, select the toggle to **ON**. When active, the device hotspot name will be displayed.





Hotspot name MC-110017

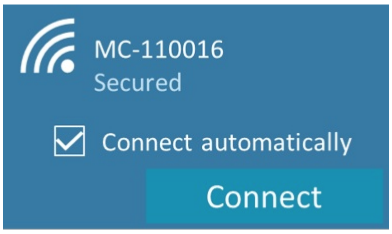
To connect the MinION Mk1C Wi-Fi router to a Windows device:

1. Locate the MinION Mk1C ID on the underside label of the device:

ONT-08-00550-00-1
OXFORD NANOPORE TECHNOLOGIES
www.nanoporetech.com
Product: MinION Mk1C
Model: MIN-101C
Input: 6.3V ═ 8.8A – 19.6V ═ 3.1A
MC-110127
Contains FCC ID: 2ARGS-P3310
Contains IC ID: 7361A-P3310
R-NZ
MAC Ethernet: 8C476E600003
(Wifi) 8C476E600004
(Bluetooth) 8C476E600005

1. Click on the MinION Mk1C ID under the **Wi-Fi Connections Panel** in the **Control Panel** or the options in the right side of the **Task Bar**

Login with the Security Key:
WarmButterflyWings98



MC-110016
Secured

☑ Connect automatically

1. Access the MinION Mk1C by the File Explorer: Go to **\\mc-XXXXXX** (XXXXXX is the MinION Mk1C ID) in the Address Bar. You will be prompted for the

1. Access the MinION Mk1C by the File Explorer: Go to **\\mc-XXXXXX** (XXXXXX is the MinION Mk1C ID) in the Address Bar. You will be prompted for the network credentials on connecting to the MinION Mk1C through File Explorer.

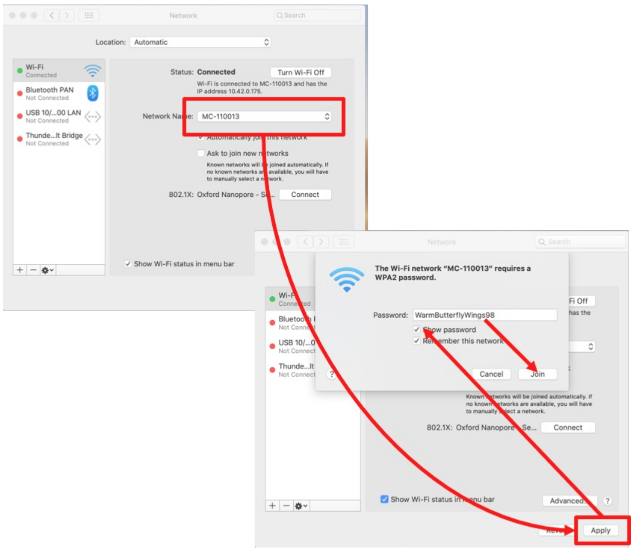
To connect the MinION Mk1C router to a macOS device:

1. Locate the MinION Mk1C ID on the underside label of the device:

1. Click on the Wi-Fi icon on the right of Menu bar at the top of the screen:

Identify the MC-XXXXXX ID in the **Network Name** under **Network Panel** (the final option under the Wi-Fi icon), which is show in the window below (left). Selecting the MinION Mk1C ID and clicking **Apply** will present the Wi-Fi login pop-up. Use the password credentials shown below.

Login with the Password:
WarmButterflyWings98


Location: Automatic

Status: Connected

Wi-Fi is connected to MC-110013 and has the IP address 10.42.0.175.

Network Name: MC-110013

☐ Ask to join new networks

Known networks will be joined automatically. If no known networks are available, you will have to manually select a network.

802.1X: Oxford Nanopore - Se...

✓ Show Wi-Fi status in menu bar

The Wi-Fi network "MC-110013" requires a WPA2 password.

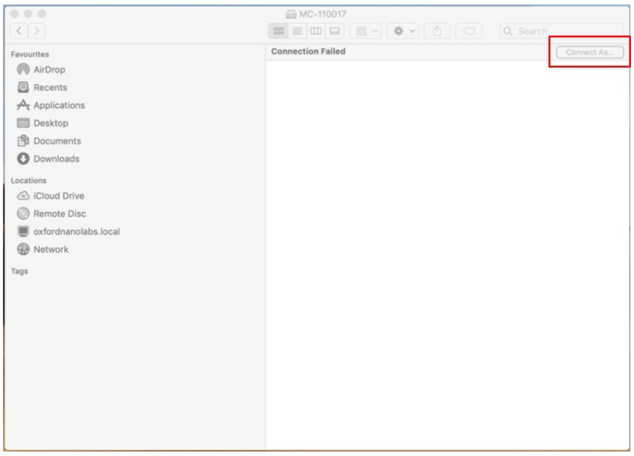
Password: WarmButterflyWings98

✓ Show password

✓ Remember this network

1. Access the MinION Mk1C by the Network browser: Search for **Network** using **Finder**. Click on **Server** in the tab to lower left of the **Search** bar.

MC-XXXXXX (XXXXXX is the MinION Mk 1C ID) will be displayed as a server option. Click the MC-XXXXXX icon and you will be prompted for the network credentials by clicking **Connect as** under the Search bar.



To connect the MinION Mk1C router to a Linux device:

1. Locate the MinION Mk1C ID on the underside label of the device:

1. Click on the Wi-Fi icon on the right of Menu bar at the top of the screen.

Identify and click on the MC-XXXXXX ID under the Wi-Fi network options icon (shown above). Enter the Wi-Fi password (shown below) in the pop-up Wi-Fi Network Authentication window:

Login with the Password:
WarmButterflyWings98

Wi-Fi Network Authentication Required

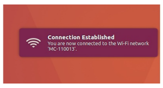
Authentication required by Wi-Fi network

Passwords or encryption keys are required to access the Wi-Fi network 'MC-110013'.

Password:

☐ Show password

The following window will be presented when you have connected to the MinION Mk1C Wi-Fi router.



Connection Established
You are now connected to the Wi-Fi network 'MC-110013'.

1. Access the MinION Mk1C using the **Linux File Browser** and enter the server address.

Open the **File Browser** (this can be found using the **Search** tool). Select **Connect to server** on the bottom of the list in the left panel. You will need to enter:

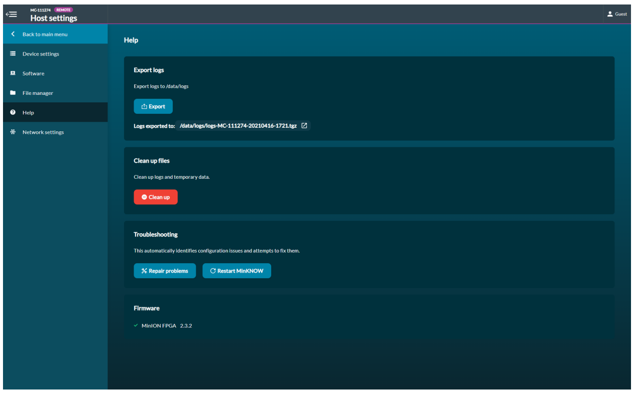
```
smb://mc-110013
```

Troubleshooting

On the Help page of host settings, troubleshooting options are available.

To export logs:

Select 'Export' in the export logs section.
When the logs are successfully exported, the user will be notified of where they are located. The logs will be downloaded as a TGZ file in the logs directory.



Export logs

Export logs to /data/logs

Logs exported to: /data/logs/logs-MC-111274-20210416-1721.tgz

Clean up files

Clean up logs and temporary data.

Troubleshooting

This automatically identifies configuration issues and attempts to fix them.

Firmware

✓ MinION FPGA 2.3.2

File manager in the host settings can be used to move the logs location where they can be easily retrieved without needing SSH.

Select the file under 'Exported logs to:' in the Export Logs panel. This will open where the logs are located in the File Manager of MinKNOW.

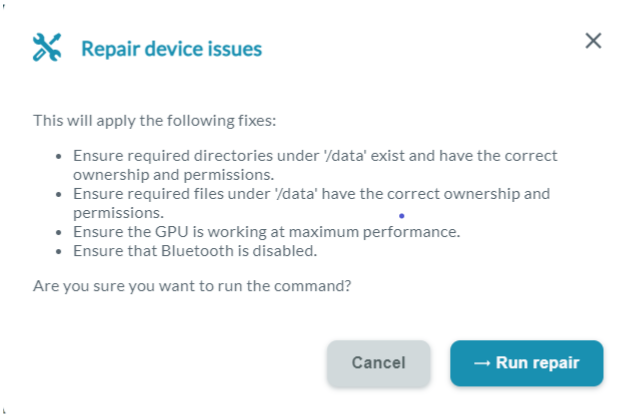
To remove logs and temporary data:

Select 'Clean up' in the clean up files section

To repair device issues:

Select 'Repair problems' in the troubleshooting section for the following fixes:

- Ensure required directories under '/data' exist and have the correct ownership and permissions
- Ensure required files under '/data' have the correct ownership and permissions
- Ensure the GPU is working at maximum performance
- Ensure that Bluetooth is disabled



This will apply the following fixes:

- Ensure required directories under '/data' exist and have the correct ownership and permissions.
- Ensure required files under '/data' have the correct ownership and permissions.
- Ensure the GPU is working at maximum performance.
- Ensure that Bluetooth is disabled.

Are you sure you want to run the command?

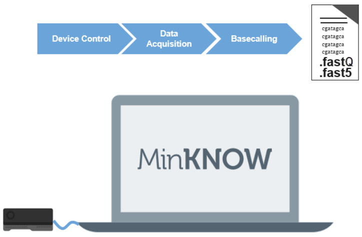
If the above fixes are required for troubleshooting, click 'Run repair' on the dialogue box to confirm.

Introduction to local basecalling

Introduction to basecalling in MinKNOW

For MinION Mk1B and Flongle (on MinION Mk1B), the MinKNOW software presents an option to basecall reads on the local computer. The basecalling is carried out live, as the read files are generated during a sequencing experiment.

Basecalling results are displayed in real-time in the MinKNOW GUI, and data is written out in the FASTQ or .fast5 file format.



The MinION Mk1C, GridION and PromethION devices have the capacity to perform live basecalling, while keeping up with one (MinION Mk1C), five (GridION) and up to 23 flow cells (PromethION P24) running fast basecalling simultaneously. Basecalling is carried out directly on the device and uses the MinKNOW software. The basecalled reads are written to the device; users can then export the reads out for further analysis via the Network or removable storage.

Starting a sequencing run with Flongle

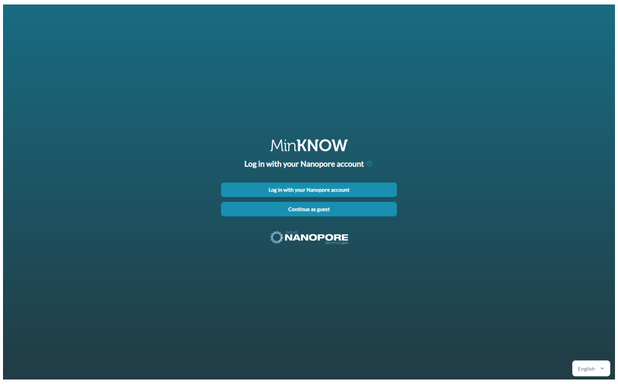
1 **Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI.**

2 **We recommend users to log into the MinKNOW software using their Community credentials.**

If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

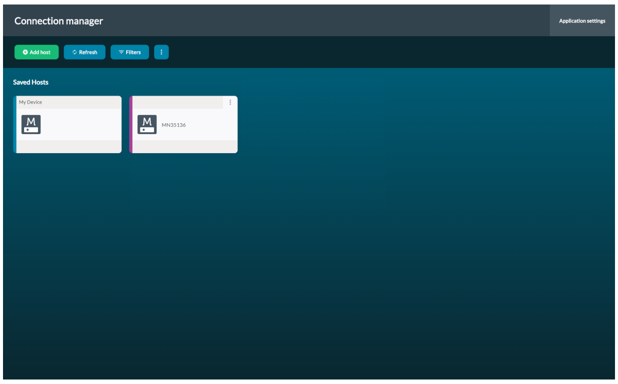
To log in, you must be connected to the internet.

From here, users may also alter language in the user interface in the bottom right corner.

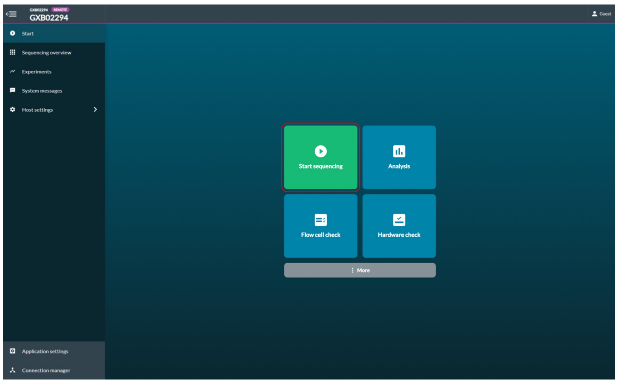


3 **If your MinION Mk1B was disconnected from the computer, plug it back in.**

4 **Select the sequencing device connected to the computer.**



5 **Select the 'Start Sequencing' option on the Start homepage to choose the running parameters for your experiment.**

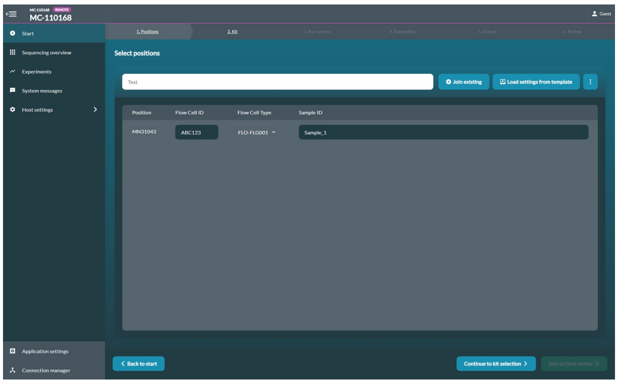


6 **Type in the experiment name, sample ID and choose flow cell type from the drop down menu. Fill in flow cell ID in the correct format: ABC123**

Ensure the experiment name and sample ID does not contain any personally identifiable information.

Note: If sample ID is not filled in, there will be no sample ID in the folder structure.

Select **Continue to Kit Selection** to move to the next page.



Select positions

Test

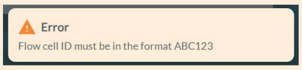
Position	Flow Cell ID	Flow Cell Type	Sample ID
MN31043	ABC123	FLO-FLG001	Sample_1

IMPORTANT

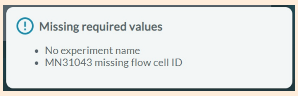
For Flongle, flow cell ID is not automatically assigned in MinKNOW. It is imperative the flow cell ID is entered in the correct format: ABC123 i.e. ([A-Z] x 3 [0-9] x 3).

The ID is case sensitive with no spaces.
The MinKNOW software will not allow you to proceed until the flow cell ID has been entered correctly.

The user will be notified in the bottom right corner of the GUI if any information entered is in the wrong format or missing.

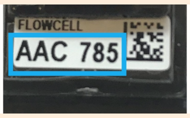


Error
Flow cell ID must be in the format ABC123



Missing required values
- No experiment name
- MN31043 missing flow cell ID

The Flongle flow cell ID is shown in the blue blox below:



TIP

Saved and default settings

To use settings saved from previous runs:

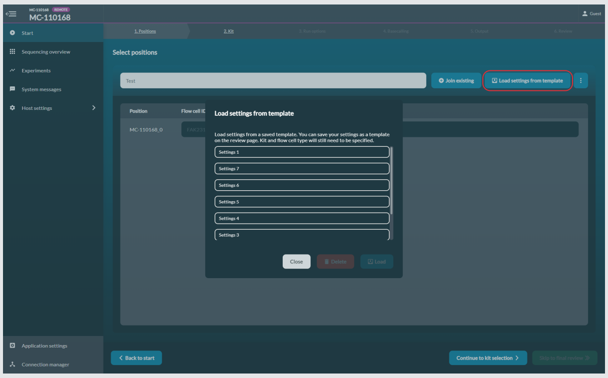
1. Click **Load settings from template**
2. Choose the saved settings to use
3. Select **Load**

Saved settings can be deleted from this window by selecting **Delete**.

Once the saved settings are loaded, the user is able to look through all options and make any changes, if needed, before starting the experiment.

The **Saved Settings** tip further down explains how to save settings.

To restore default run settings, select the three dots and select **Reload Scripts**.



Load settings from template

Load settings from a saved template. You can save your settings as a template on the review page. Kit and flow cell type will still need to be specified.

- Settings 1
- Settings 7
- Settings 6
- Settings 5
- Settings 4
- Settings 3

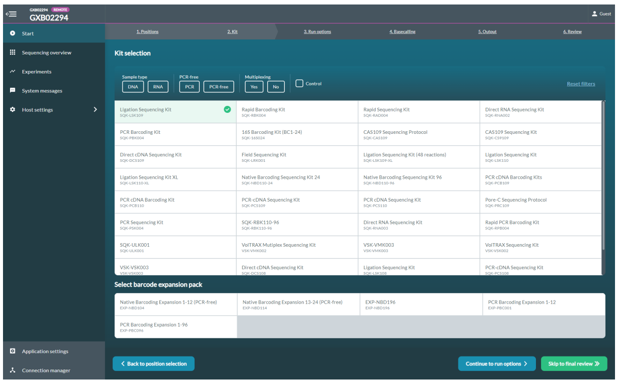
7 **Select the kit used from the Kit selection menu.**

The filter options may be used to find the kit used. For example, if running a Control Experiment, check the **Control** box on the right.
Note: The five most popular kits are displayed in the top rows.

Demultiplexing is referred to as barcoding in this section. If you have used a barcoding kit/expansion pack for your library preparation, the demultiplexing option in MinKNOW can be used to split your reads by barcode without having to use command line-tools. Demultiplexing places reads into barcode-specific folders.

Once a kit is selected, choose the appropriate option from **Select Barcode Expansion Pack** options if a barcode expansion pack has been used.
Note: Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear.

Select **Continue to Run Options** to choose run parameters.



Ligation Sequencing Kit ✓ SQK-LSK109	Rapid Barcoding Kit SQK-RBK004	Rapid Sequencing Kit SQK-RAD004	Direct RNA Sequencing Kit SQK-RNA002
PCR Barcoding Kit SQK-PBK004	16S Barcoding Kit (BC1-24) SQK-16S024	CAS109 Sequencing Protocol SQK-CAS109	CAS109 Sequencing Kit SQK-CS9109
Direct cDNA Sequencing Kit SQK-DCS109	Field Sequencing Kit SQK-LRK001	Ligation Sequencing Kit (48 reactions) SQK-LSK109-XL	Ligation Sequencing Kit SQK-LSK110
Ligation Sequencing Kit XL SQK-LSK110-XL	Native Barcoding Sequencing Kit 24 SQK-NBD110-24	Native Barcoding Sequencing Kit 96 SQK-NBD110-96	PCR cDNA Barcoding Kits SQK-PCB109
PCR cDNA Barcoding Kit SQK-PCB110	PCR-cDNA Sequencing Kit SQK-PCS109	PCR cDNA Sequencing Kit SQK-PCS110	Pore-C Sequencing Protocol SQK-PRC109
PCR Sequencing Kit SQK-PSK004	SQK-RBK110-96 SQK-RBK110-96	Direct RNA Sequencing Kit SQK-RNA003	Rapid PCR Barcoding Kit SQK-RPB004
SQK-ULK001 SQK-ULK001	VolTRAX Mutiplex Sequencing Kit VSK-VMK002	VSK-VMK003 VSK-VMK003	VolTRAX Sequencing Kit VSK-VSK002
VSK-VSK003 VSK-VSK003	Direct cDNA Sequencing Kit SQK-DCS108	Ligation Sequencing Kit SQK-LSK108	PCR-cDNA Sequencing Kit SQK-PCS108

Select barcode expansion pack

Native Barcoding Expansion 1-12 (PCR-free) EXP-NBD104	Native Barcoding Expansion 13-24 (PCR-free) EXP-NBD114	EXP-NBD196 EXP-NBD196	PCR Barcoding Expansion 1-12 EXP-PBC001
PCR Barcoding Expansion 1-96 EXP-PBC096			

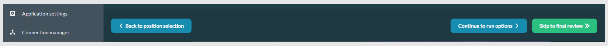
TIP

Skip to final review

To skip to the end of Start Sequencing, select **Skip to final review**.

The user is also able to return to any previous pages by using the **Back to** button.

We recommend users keep the default settings.



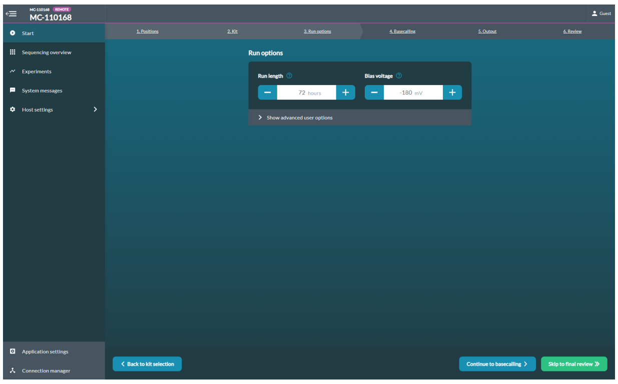
8 **Edit run length and bias voltage in Run Options.**

The run options tab provides variables for run time and starting voltage. By default, these will be 72 hours and -180mV. However, a run can be manually stopped early by the user.

Select and type or use the **+** and **—** to increase or decrease settings.

For more information about adjusting starting voltage, refer to Adjusting the starting potential in this protocol.

Select **Continue to Basecalling** to proceed to the next section.



Run options

Run length: 72 hours

Bias voltage: -180 mV

TIP

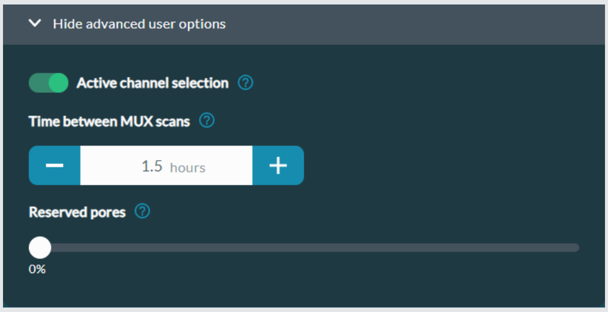
Advanced User Options

Options to turn active channel selection either on or off.

Active channel selection refers to a feature whereby if a pore is in the 'Saturated' or 'Multiple' state, the software instantly switches to a new pore in the group. If a pore is 'Recovering', MinKNOW will attempt to revert the pore back to 'Pore' or 'Sequencing' for ~5 minutes, after which it will select a new pore in the group. This maximises the number of pores sequencing at the start of the experiment.

Time between Mux scans and percentage of reserved pores can be altered to maximise pores for sequencing.

Percentage of reserved pores may also be selected here.



Active channel selection

Time between MUX scans: 1.5 hours

Reserved pores: 0%

9 **Choose basecalling, barcoding and alignment options.**

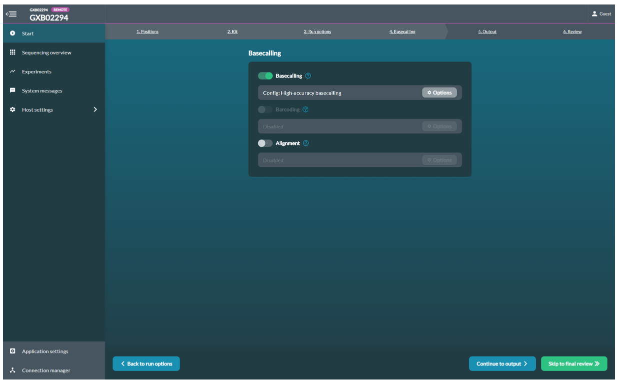
Barcoding options are only available when a barcoding expansion pack has been selected.

If the user does not wish to demultiplex, unselect the toggle to disable barcoding.

To use alignment during sequencing, select the toggle and upload an alignment reference file as a .fasta or minimap index file.

Note: For barcoding or alignment to be performed during sequencing, basecalling *must* be enabled.

Select **Continue to Output** to proceed.



Config: High-accuracy basecalling

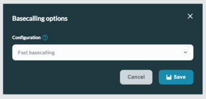
TIP

User options

Use the **Options** button to further customise a run:

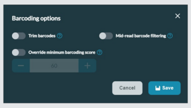
1. Choose basecall model
2. Trim barcodes or alter barcoding score
    - Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
    - Barcoding score can be increased if the user wants to be more confident in barcode detection classification.
3. Enter an alignment reference
    - We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.
    - A reference file must be uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes). Alignment hits from these files are used to populate the alignment graphs.
    - A .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in a chromosome). Alignment hits from .bed files will be highlighted in the sequencing .txt file generated in the data folder.

1.

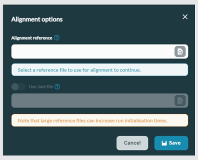


Fast basecalling

2.



3.



Select a reference file to use for alignment to continue.

Note that large reference files can increase run initialisation times.

Each barcode demultiplexed by the basecaller will have its own folder and can be processed separately:

```
/data/fastq_pass/barcode[01-XX]
/data/fastq-fail/barcode[01-XX]
```

Note: Barcoding and alignment options are only available when basecalling is enabled.

For more information regarding barcoding, demultiplexing, and alignment, please refer to the Guppy protocol.

Basecall models

Basecalling models can be selected at two stages in the MinKNOW software:

- **Setting up an experiment in MinKNOW** - the instructions for selecting a model can be found in the section 'Starting a sequencing run' of this protocol.
- **Using post-run basecalling in MinKNOW** - the instructions for how to basecall data once an experiment has finished can be found in the section 'Post-run analysis' of this protocol.

Guppy, the basecaller in MinKNOW, provides multiple models for basecalling nanopore data.

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

1. Fast basecalling - This model is able to keep up with a high-throughput sequencing experiment on a MinION Mk1B, MinION Mk1C, GridION or PromethION. This delivers up to 95.8% single-molecule accuracy.

2. High-accuracy basecalling (HAC) - This provides a higher (up to 97.8%) single-molecule accuracy than the Fast model. It is currently 5-8 times more computationally-intensive than the Fast model, so users should ensure their data transfer and device utilisation is scaled appropriately for this.

3. Super Accurate basecalling (SUP) - The Super accurate model has an even higher single-molecule accuracy (up to 98.3%), and is ~3 times more intensive than the HAC model.

4. Modified basecalling for 5mC - This model is provided in MinKNOW and allows the user to basecall certain modified bases (currently 5mC). To understand how the basecaller assigns the basecalled modifications, please read the Stand-alone Guppy protocol, under Output - Modified basecalling.

4. No basecalling

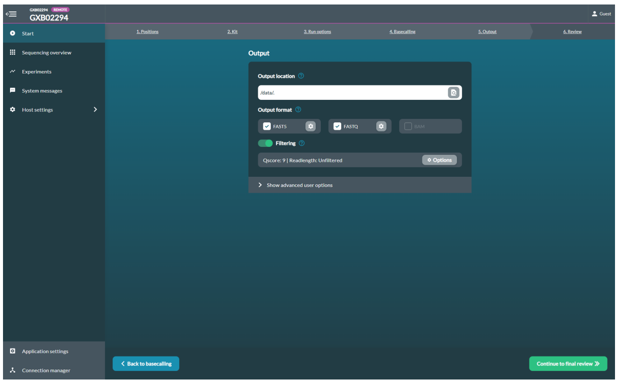
Note: GPU devices will basecall in near real-time; CPU devices, like a standard laptop for MinION Mk1B, will not maintain real-time basecalling.

10 Select the output data location, format and filtering options.

Use the checkboxes to save output data as FAST5, FASTQ and/or BAM files.
An alternative location can be selected to which processed reads will be written out by using **Output Location**.

Filtering options can be used to determine which reads are classed into pass or fail files. These options may also be used to determine which predefined reads, read lengths and Qscore during basecalling can be split out in some live graphs.

Select **Continue to final review** to proceed.



Output location: /data/.

Output format: ☑ FAST5 ☑ FASTQ ☐ BAM

Filtering: Qscore: 9 | Readlength: Unfiltered

Further options:

Select **Options** in the Output Format panel of the Output tab.

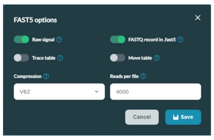
- FAST5: The number of basecalls that MinKNOW will write in a single file.
  - By default, all reads are written out per file for Flongle
  - The files can be compressed with zlib or VBZ (note: VBZ compression will require a VBZ plugin for compatibility with existing tools. This is optimised for nanopore data, with improved compression over zlib)
- FASTQ: The number of files that MinKNOW will write to a single folder.
  - By default, all files are written into a single folder for Flongle
  - The files can be uncompressed, or compressed with Gzip to ~55% of original size
- BAM: Files containing aligned reads (one BAM file per input FASTQ file).

Users can change reads per file, but take note:

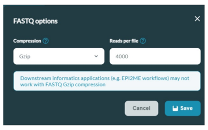
- Fewer reads per file: reads become more quickly available. However, too few reads per file means MinKNOW may not keep up with writing out files in real-time.
- More reads per file: reduces the number of files especially for amplicon/cDNA experiments that produce a large number of reads. Some downstream analysis tools may have an upper limit on the uploaded file size.

Filtering options allows the user to alter Qscore and minimum and maximum read length.

Chosen options can be saved by selecting **Save** for future use.

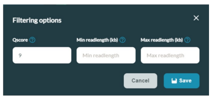


Raw signal: on
FASTQ record in .fast5: on
Trace table: off
Move table: off
Compression: VBZ
Reads per file: 4000



Compression: Gzip
Reads per file: 4000

Downstream informatics applications (e.g. EPI2ME workflows) may not work with FASTQ Gzip compression



Qscore: 9
Min readlength (kb): Min readlength
Max readlength (kb): Max readlength

TIP

Advanced User Options

Option to turn bulk file saving either on or off.

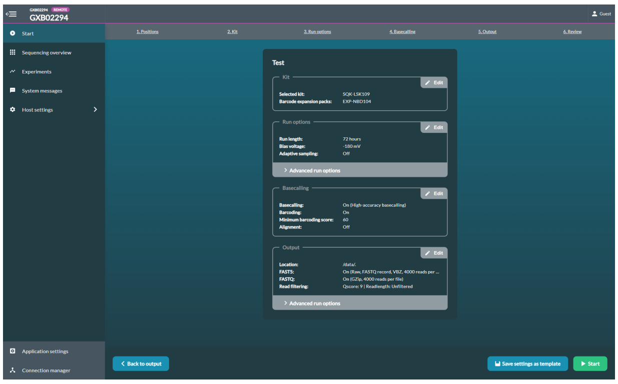
Note: This will result in much larger file sizes due to additional information about the run, which is used for debugging.

11 **Click 'Start' to run the experiment.**

The **Review** page is an overview of all run options selected.

Edits can be made by selecting the **Edit** button.

Select **Advanced run options** to view the extra options selected.



Test

Kit

Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options

Run length: 72 hours
Bias voltage: -180 mV
Adaptive sampling: Off

Basecalling

Basecalling: On (High-accuracy basecalling)
Barcoding: On
Minimum barcoding score: 60
Alignment: Off

Output

Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

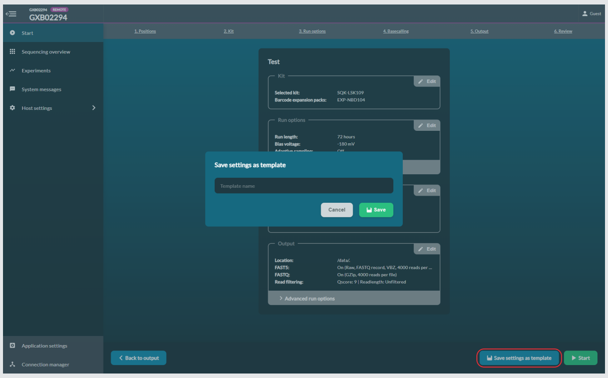
TIP

Save settings

Save settings to reload in following runs as outlined in the **Saved and default settings** tip.

Select **Save settings as template** once all settings have been chosen.

Fill in settings name and select **Save**.



Test

Kit

Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options

Run length: 72 hours
Bias voltage: -180 mV

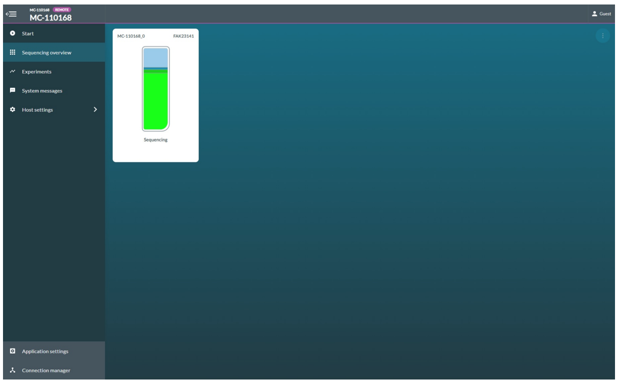
Output

Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

12 Users will be automatically navigated to the Sequencing Overview when sequencing starts.

From here, users can see a progression bar below the flow cell to show the progression of the sequencing script.

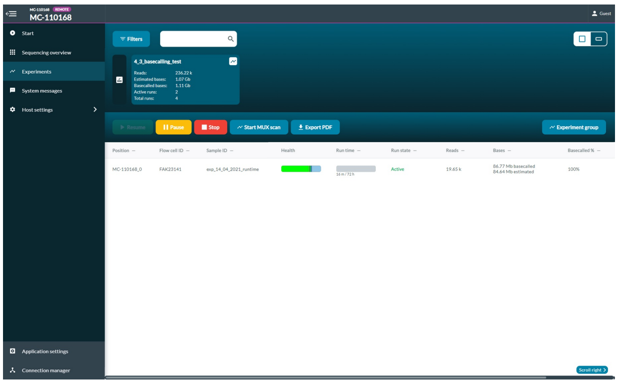
Flow cell health will be displayed after the first Mux scan.



13 Select the flow cell to open the quick view and check the number of active pores. The first Mux scan should report a similar number of active pores (within 10-15%) to that reported in the flow cell check.

- If there is a significant reduction in active pores in the first Mux scan, restart MinKNOW.
- If the numbers are still significantly different, close down the host computer and reboot.
- When the numbers are similar to those reported at the end of the flow cell check, restart the experiment. There is no need to load any additional library after the restart.

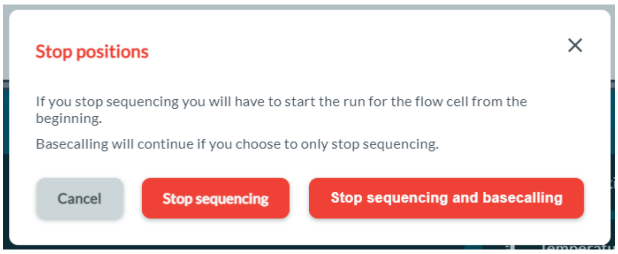
14 To stop the experiment, select 'Stop' on the experiments page.



4_3_basecalling_test
Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
MC-110168_0	FAK23141	exp_14_04_2021_runtime		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%

15 In the new window, select either 'Stop sequencing' or 'Stop sequencing and basecalling'.



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

Note: If you select 'Stop sequencing' any catch-up basecalling will still occur. A progress bar will appear on associated flow cell on the Sequencing Overview tab.

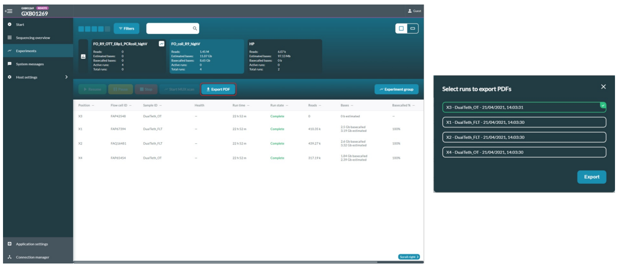
16 **Once sequencing is complete, generate a run report.**

A PDF report containing information about the sequencing run and performance graphs can be generated by clicking **Export PDF**.

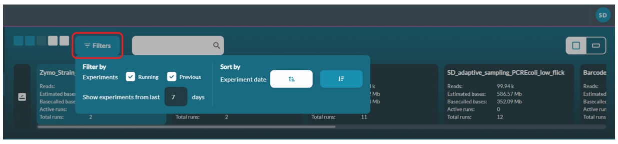
PDF reports are automatically generated for MinION Mk1C, GridION and PromethION experiments. For other platforms (e.g. MinION Mk1B running on a laptop), the PDF reports need to be manually generated. Click **Export PDF** and select which experiment to export PDF.

A duty time CSV file is also generated for every run.

The report PDF and CSV files are saved to the same folder as the .fast5 and .fastq read files e.g. :\data\experiment\sample_ID\ for MinION Mk1B.



To access previous sequencing runs and view them on the UI, click 'Filters' and select how many days previous to show past experiments.



END OF STEP

MinKNOW fallback folder

If writing out reads to your specified location fails (e.g. if the disk becomes full, or the connection is disrupted), the experiment will continue, and the reads will be written into a fallback folder:

Windows

.fast5 files: C:\data\reads\fallback (with the standard run data name sample ID as a folder)
FASTQ files: C:\data\reads\fastq_fallback (with the standard run data name sample ID as a folder)

Mac OS X:

.fast5 files: /Library/MinKNOW/data/fallback
FASTQ files: /Library/MinKNOW/data/fastq_fallback

Linux:

.fast5 files: /var/lib/MinKNOW/data/fallback
FASTQ files: /var/lib/MinKNOW/data/fastq_fallback

Starting a sequencing run on MinION Mk1B

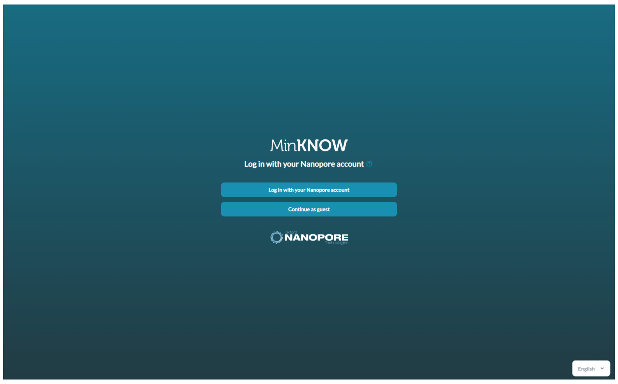
1 **Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI.**

2 **We recommend users to log into the MinKNOW software using their Community credentials.**

If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

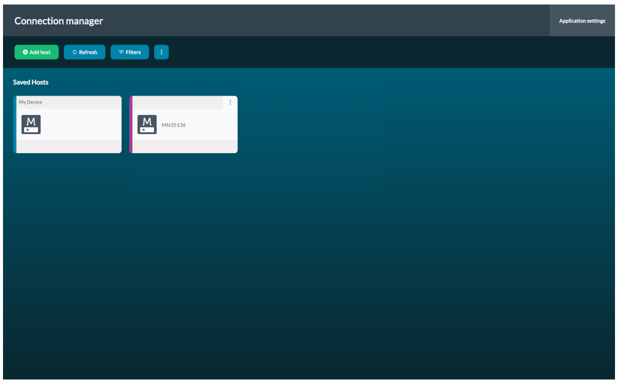
To log in, you must be connected to the internet.

From here, users may also alter language in the user interface in the bottom right corner.

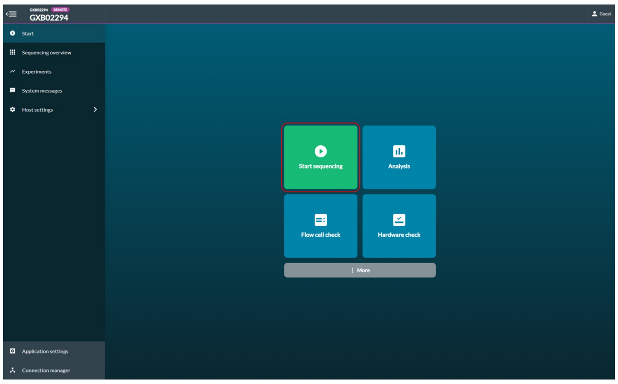


3 **If your MinION Mk1B was disconnected from the computer, plug it back in.**

4 **Select the sequencing device connected to the computer.**



5 **Select the 'Start Sequencing' option on the Start homepage to choose the running parameters for your experiment.**

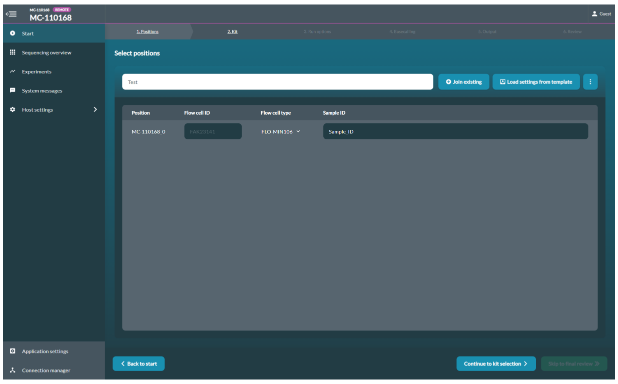


6 **Type in the experiment name, sample ID and choose flow cell type from the drop down menu.**

Ensure the experiment name and sample ID does not contain any personally identifiable information.

Note: If sample ID is not filled in, there will be no sample ID in the folder structure.

Select **Continue to Kit Selection** to move to the next page.



Select positions

Test

Position	Flow cell ID	Flow cell type	Sample ID
MC-110168_0	FAK23141	FLO-MIN106	Sample_ID

TIP

Saved and default settings

To use settings saved from previous runs:

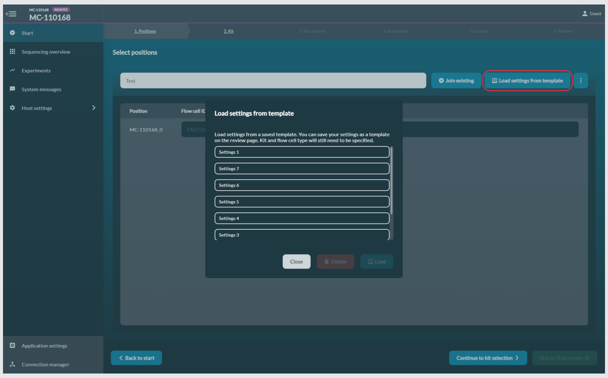
1. Click **Load settings from template**
2. Choose the saved settings to use
3. Select **Load**

Saved settings can be deleted from this window by selecting **Delete**.

Once the saved settings are loaded, the user is able to look through all options and make any changes, if needed, before starting the experiment.

The **Saved Settings** tip further down explains how to save settings.

To restore default run settings, select the three dots and select **Reload Scripts**.



Select positions

Test

Load settings from template

Load settings from a saved template. You can save your settings as a template on the review page. Kit and flow cell type will still need to be specified.

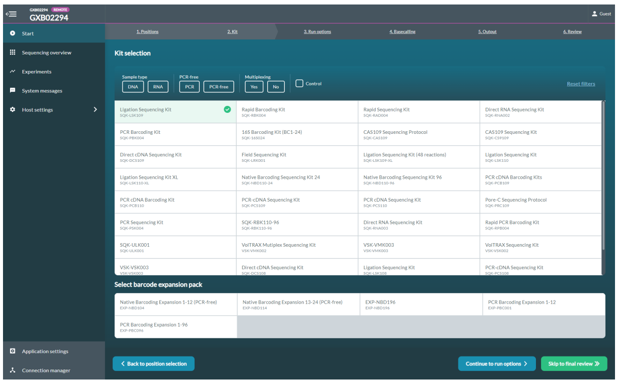
7 **Select the kit used from the Kit selection menu.**

The filter options may be used to find the kit used. For example, if running a Control Experiment, check the **Control** box on the right.
Note: The five most popular kits are displayed in the top rows.

Demultiplexing is referred to as barcoding in this section. If you have used a barcoding kit/expansion pack for your library preparation, the demultiplexing option in MinKNOW can be used to split your reads by barcode without having to use command line-tools. Demultiplexing places reads into barcode-specific folders.

Once a kit is selected, choose the appropriate option from **Select Barcode Expansion Pack** options if a barcode expansion pack has been used.
Note: Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear.

Select **Continue to Run Options** to choose run parameters.



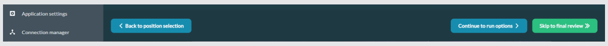
TIP

Skip to final review

To skip to the end of Start Sequencing, select **Skip to final review**.

The user is also able to return to any previous pages by using the **Back to** button.

We recommend users keep the default settings.



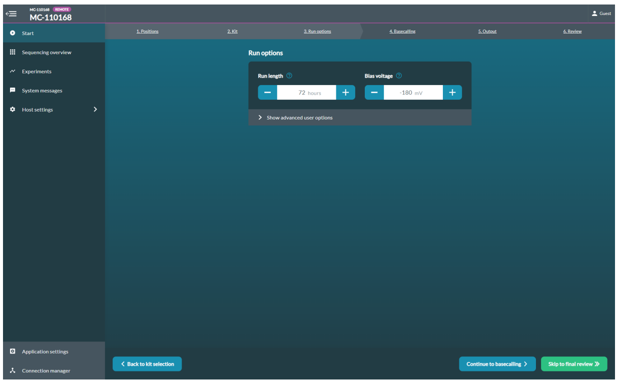
8 **Edit run length and bias voltage in Run Options.**

The run options tab provides variables for run time and starting voltage. By default, these will be 72 hours and -180mV. However, a run can be manually stopped early by the user.

Select and type or use the **+** and **—** to increase or decrease settings.

For more information about adjusting starting voltage, refer to Adjusting the starting potential in this protocol.

Select **Continue to Basecalling** to proceed to the next section.



Run length — 72 hours +
Bias voltage — -180 mV +

Optional Action
Adaptive sampling

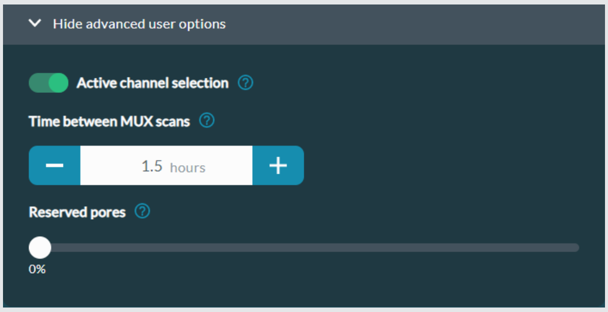
Adaptive sampling is available on MinION Mk1B. However, users must enable live GPU basecalling on a Linux computer using a NVIDIA GPU by following these instructions.

This is done at the user's own risk:

- mis-configuration of the GPU may result in slow basecalling
- and/or a large number of skipped reads if the basecall server crashes as a result of mis-parameterisation

  - GPUs with a low amount of memory (<4 GB) may not work.

> **TIP**
>
> **Advanced User Options**
>
> Options to turn active channel selection either on or off.
>
> Active channel selection refers to a feature whereby if a pore is in the 'Saturated' or 'Multiple' state, the software instantly switches to a new pore in the group. If a pore is 'Recovering', MinKNOW will attempt to revert the pore back to 'Pore' or 'Sequencing' for ~5 minutes, after which it will select a new pore in the group. This maximises the number of pores sequencing at the start of the experiment.
>
> Time between Mux scans and percentage of reserved pores can be altered to maximise pores for sequencing.
>
> Percentage of reserved pores may also be selected here.



Active channel selection

Time between MUX scans: 1.5 hours

Reserved pores: 0%

9 **Choose basecalling, barcoding and alignment options.**

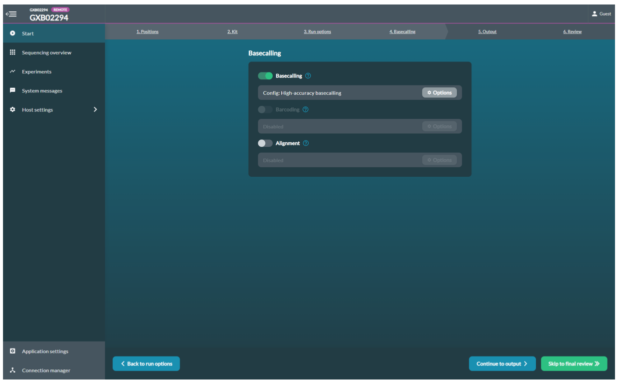
Barcoding options are only available when a barcoding expansion pack has been selected.

If the user does not wish to demultiplex, unselect the toggle to disable barcoding.

To use alignment during sequencing, select the toggle and upload an alignment reference file as a .fasta or minimap index file.

Note: For barcoding or alignment to be performed during sequencing, basecalling *must* be enabled.

Select **Continue to Output** to proceed.



Basecalling

Basecalling: Config: High-accuracy basecalling — Options

Barcoding: Disabled — Options

Alignment: Disabled — Options

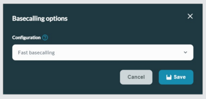
TIP

User options

Use the **Options** button to further customise a run:

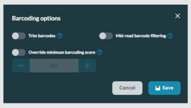
1. Choose basecall model
2. Trim barcodes or alter barcoding score
    - Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
    - Barcoding score can be increased if the user wants to be more confident in barcode detection classification.
3. Enter an alignment reference
    - We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.
    - A reference file must be uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes). Alignment hits from these files are used to populate the alignment graphs.
    - A .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in a chromosome). Alignment hits from .bed files will be highlighted in the sequencing .txt file generated in the data folder.

1.

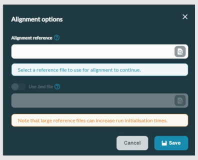


Configuration: Fast basecalling

2.



3.



Select a reference file to use for alignment to continue.

Note that large reference files can increase run initialisation times.

Each barcode demultiplexed by the basecaller will have its own folder and can be processed separately:

```
/data/fastq_pass/barcode[01-XX]
/data/fastq-fail/barcode[01-XX]
```

Note: Barcoding and alignment options are only available when basecalling is enabled.

For more information regarding barcoding, demultiplexing, and alignment, please refer to the Guppy protocol.

Basecall models

Basecalling models can be selected at two stages in the MinKNOW software:

- **Setting up an experiment in MinKNOW** - the instructions for selecting a model can be found in the section 'Starting a sequencing run' of this protocol.
- **Using post-run basecalling in MinKNOW** - the instructions for how to basecall data once an experiment has finished can be found in the section 'Post-run analysis' of this protocol.

Guppy, the basecaller in MinKNOW, provides multiple models for basecalling nanopore data.

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

1. Fast basecalling - This model is able to keep up with a high-throughput sequencing experiment on a MinION Mk1B, MinION Mk1C, GridION or PromethION. This delivers up to 95.8% single-molecule accuracy.

2. High-accuracy basecalling (HAC) - This provides a higher (up to 97.8%) single-molecule accuracy than the Fast model. It is currently 5-8 times more computationally-intensive than the Fast model, so users should ensure their data transfer and device utilisation is scaled appropriately for this.

3. Super Accurate basecalling (SUP) - The Super accurate model has an even higher single-molecule accuracy (up to 98.3%), and is ~3 times more intensive than the HAC model.

4. Modified basecalling for 5mC - This model is provided in MinKNOW and allows the user to basecall certain modified bases (currently 5mC). To understand how the basecaller assigns the basecalled modifications, please read the Stand-alone Guppy protocol, under Output - Modified basecalling.

4. No basecalling

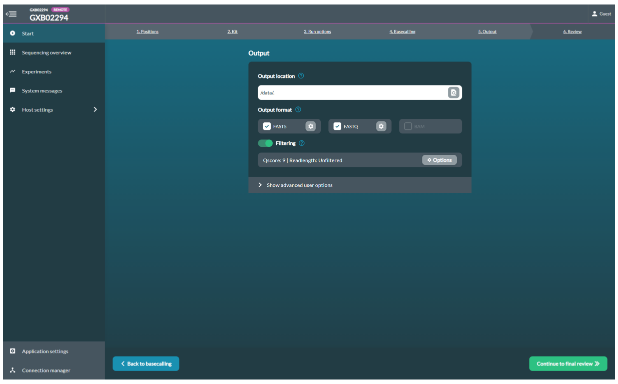
Note: GPU devices will basecall in near real-time; CPU devices, like a standard laptop for MinION Mk1B, will not maintain real-time basecalling.

10 Select the output data location, format and filtering options.

Use the checkboxes to save output data as FAST5, FASTQ and/or BAM files.
An alternative location can be selected to which processed reads will be written out by using **Output Location**.

Filtering options can be used to determine which reads are classed into pass or fail files. These options may also be used to determine which predefined reads, read lengths and Qscore during basecalling can be split out in some live graphs.

Select **Continue to final review** to proceed.



Output

Output location: /data/.

Output format: ☑ FAST5 ☑ FASTQ ☐ BAM

Filtering

Qscore: 9 | Readlength: Unfiltered

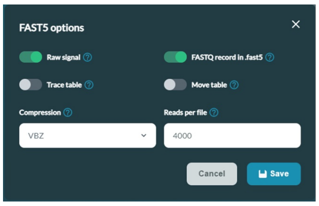
Further options:

Select **Options** in the Output Format panel of the Output tab.

- FAST5: The number of basecalls that MinKNOW will write in a single file.
  - By default, 4000 reads are written out per file
  - The files can be compressed with zlib or VBZ (note: VBZ compression will require a VBZ plugin for compatibility with existing tools. This is optimised for nanopore data, with improved compression over zlib)
- FASTQ: The number of files that MinKNOW will write in a single folder.
  - By default, 4000 files are written into a single folder
  - The files can be uncompressed, or compressed with Gzip to ~55% of original size
- BAM: Files containing aligned reads (one BAM file per input FASTQ file).

Users can change reads per file:

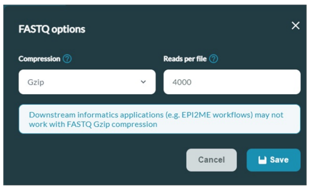
- Fewer reads per file: reads become more quickly available. However, too few reads per file means MinKNOW may not keep up with writing out files in real-time.
- More reads per file: reduces the number of files especially for amplicon/cDNA experiments that produce a large number of reads. Some downstream analysis tools may have an upper limit on the uploaded file size.



FAST5 options

Compression: VBZ

Reads per file: 4000



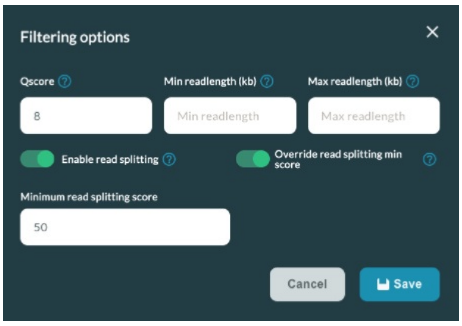
FASTQ options

Compression: Gzip

Reads per file: 4000

Downstream informatics applications (e.g. EPI2ME workflows) may not work with FASTQ Gzip compression

Filtering options allows the user to alter Qscore and mid and max read length. Users can also enable read splitting which is a tool used to classify concatermerised reads as "informatic chimeras" for our Kit 12 chemistry. For more information, please see the Kit 12 device and informatics info sheet .



Filtering options

Qscore: 8

Min readlength (kb): Min readlength

Max readlength (kb): Max readlength

Enable read splitting

Override read splitting min score

Minimum read splitting score: 50

Chosen options can be saved by selecting **Save** for future use.

TIP

Advanced User Options

Option to turn bulk file saving either on or off.

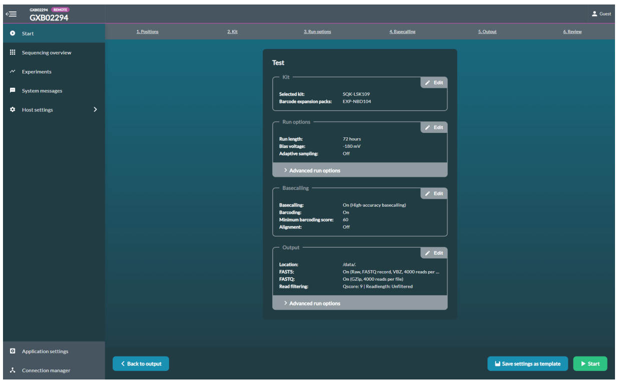
Note: This will result in much larger file sizes due to additional information about the run, which is used for debugging.

11 Click 'Start' to run the experiment.

The **Review** page is an overview of all run options selected.

Edits can be made by selecting the **Edit** button.

Select **Advanced run options** to view the extra options selected.



Test

Kit

Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options

Run length: 72 hours
Bias voltage: -180 mV
Adaptive sampling: Off

Advanced run options

Basecalling

Basecalling: On (High-accuracy basecalling)
Barcoding: On
Minimum barcoding score: 60
Alignment: Off

Output

Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

Advanced run options

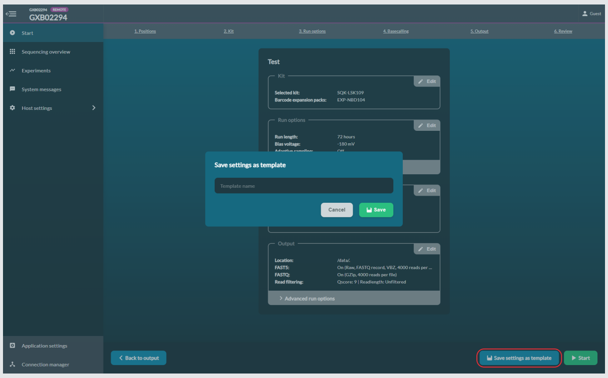
TIP

Save settings

Save settings to reload in following runs as outlined in the **Saved and default settings** tip.

Select **Save settings as template** once all settings have been chosen.

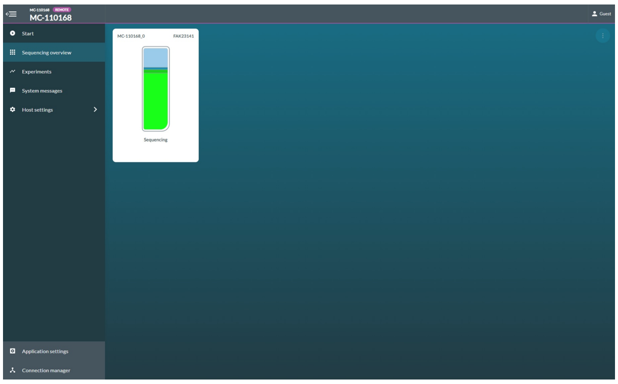
Fill in settings name and select **Save**.



12 Users will be automatically navigated to the Sequencing Overview when sequencing starts.

From here, users can see a progression bar below the flow cell to show the progression of the sequencing script.

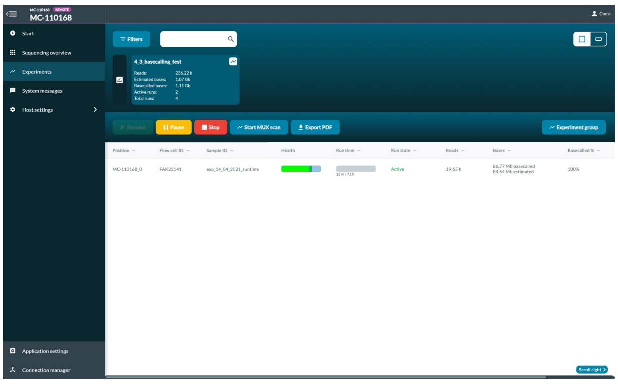
Flow cell health will be displayed after the first Mux scan.



13 Select the flow cell to open the quick view to check the number of active pores reported in the Mux scan are similar (within 10-15%) to those reported at the end of the Flow Cell Check.

- If there is a significant reduction in the numbers, restart MinKNOW.
- If the numbers are still significantly different, close down the host computer and reboot.
- When the numbers are similar to those reported at the end of the Flow Cell Check, restart the experiment. There is no need to load any additional library after restart.

14 To stop the experiment, select 'Stop' on the experiments page.

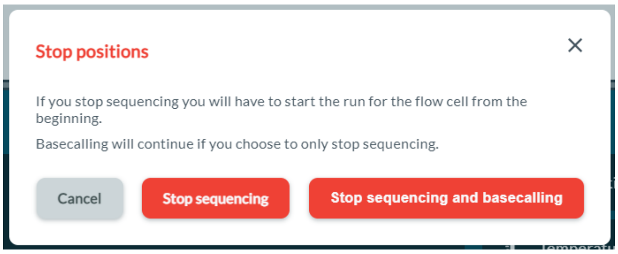


4_3_basecalling_test

Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
MC-110168_0	FAK23141	exp_14_04_2021_runtime		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%

15 In the new window, select either 'Stop sequencing' or 'Stop sequencing and basecalling'.



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

Note: If you select 'Stop sequencing' any catch-up basecalling will still occur. A progress bar will appear on associated flow cell on the Sequencing Overview tab.

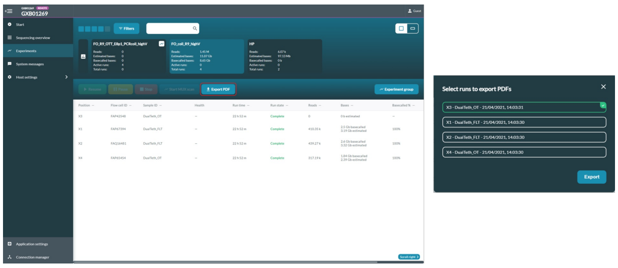
16 **Once sequencing is complete, generate a run report.**

A PDF report containing information about the sequencing run and performance graphs can be generated by clicking **Export PDF**.

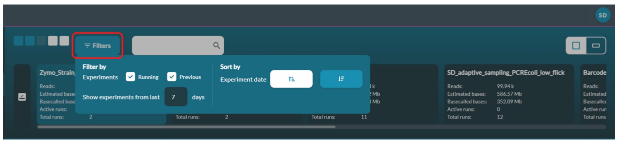
PDF reports are automatically generated for MinION Mk1C, GridION and PromethION experiments. For other platforms (e.g. MinION Mk1B running on a laptop), the PDF reports need to be manually generated. Click **Export PDF** and select which experiment to export PDF.

A duty time CSV file is also generated for every run.

The report PDF and CSV files are saved to the same folder as the .fast5 and .fastq read files e.g. :\data\experiment\sample_ID\ for MinION Mk1B.



To access previous sequencing runs and view them on the UI, click 'Filters' and select how many days previous to show past experiments.



END OF STEP

MinKNOW fallback folder

If writing out reads to your specified location fails (e.g. if the disk becomes full, or the connection is disrupted), the experiment will continue, and the reads will be written into a fallback folder:

Windows

.fast5 files: C:\data\reads\fallback (with the standard run data name sample ID as a folder)
FASTQ files: C:\data\reads\fastq_fallback (with the standard run data name sample ID as a folder)

Mac OS X:

.fast5 files: /Library/MinKNOW/data/fallback
FASTQ files: /Library/MinKNOW/data/fastq_fallback

Linux:

.fast5 files: /var/lib/MinKNOW/data/fallback
FASTQ files: /var/lib/MinKNOW/data/fastq_fallback

Starting a sequencing run on MinION Mk1C

There are two versions of the MinKNOW GUI on the MinION Mk1C

The MinKNOW GUI on the device:

The version of the Graphical User Interface (GUI) described in this document is the touch-screen configurable GUI.

The MinKNOW GUI via Wi-Fi or Ethernet connection from a laptop:

The GUI presented on the laptop when connected to the MinION Mk1C is the same as MinION Mk1B desktop MinKNOW GUI. See the MinION Mk1C protocol for how to use the Wi-Fi or wired connection from a device to the MinION Mk1C version of the MinKNOW GUI.

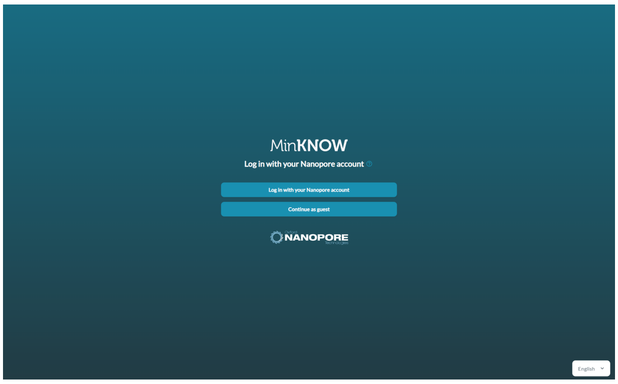
1 **Turn on the Mk1C or double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI to open MinKNOW on a laptop.**

2 **We recommend users to log into the MinKNOW software using their Community credentials.**

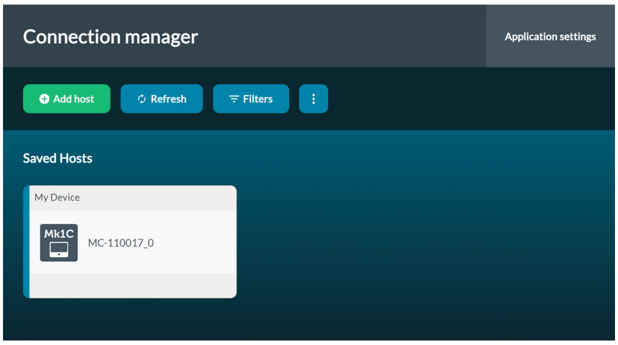
If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

To log in, you must be connected to the internet.

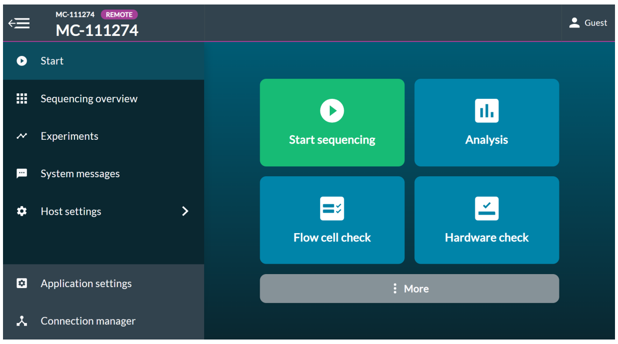
From here, users may also alter language in the user interface in the bottom right corner.



3 **Select the sequencing device connected to the computer.**



4 **Select the 'Start Sequencing' option on the Start homepage to choose the running parameters for your experiment.**

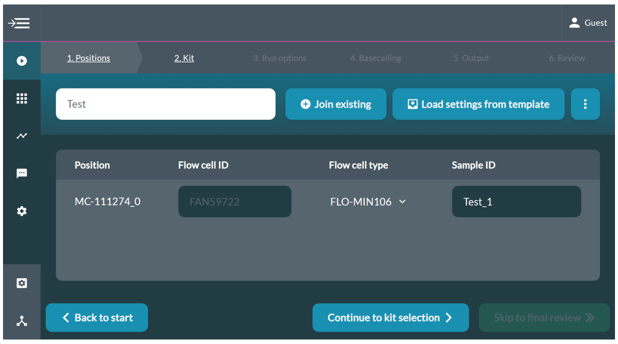


5 **Type in experiment name, sample ID and choose flow cell type from the drop down menu.**

Ensure the experiment name and sample ID does not contain any personally identifiable information.

Note: If sample ID is not filled in, there will be no sample ID in the folder structure.

Select **Continue to Kit Selection** to move to the next page.



Test

Position	Flow cell ID	Flow cell type	Sample ID
MC-111274_0	FAN59722	FLO-MIN106	Test_1

TIP

Saved and default settings

To used settings saved from previous runs:

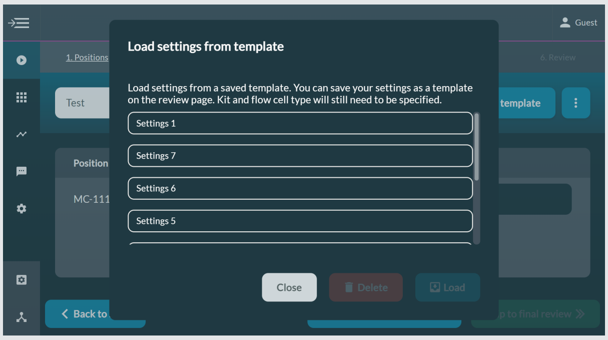
1. Select **Load Settings from template**
2. Choose the saved settings to use
3. Select **Load**.

Saved settings can be deleted from this window by selecting **Delete**.

Once the saved settings are loaded, the user is able to look through all options and make any changes, if needed, before starting the experiment.

The **Save Settings** tip further down explains how to save settings.

To restore default run settings, select the three dots and select **Reload Scripts**.



Load settings from a saved template. You can save your settings as a template on the review page. Kit and flow cell type will still need to be specified.

6 **Select the kit used from the Kit selection menu.**

The filter options can be used to find the kit used. If running a control experiment, check the **Control** box on the right.

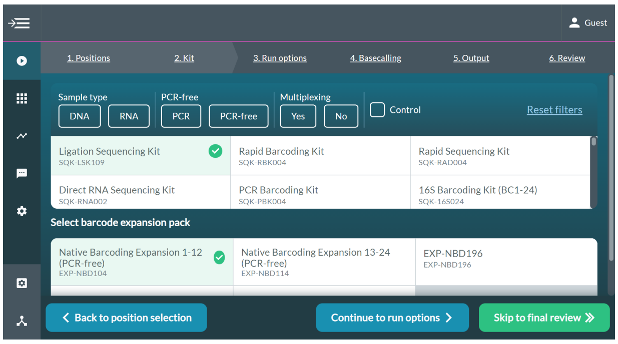
Note: The five most popular kits are displayed in the top rows.

Demultiplexing is referred to as barcoding in this section and the MinKNOW GUI. If you have used a barcoding kit/expansion pack for your library preparation, the demultiplexing option in MinKNOW can be used to split your reads by barcode without having to use command line-tools. Demultiplexing places reads into barcode-specific folders.

Once a kit is selected, choose the appropriate option(s) from **Select Barcode Expansion Pack** options if a barcode expansion pack has been used.

Note: Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear.

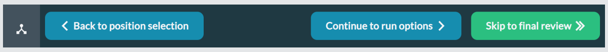
Select **Continue to Run Options** to choose run parameters.



TIP

Skip to final review

To skip to the end of Start Sequencing, select **Skip to final review**.
The user is also able to return to any previous pages by using the **Back to** button.
We recommend users to keep the default settings.

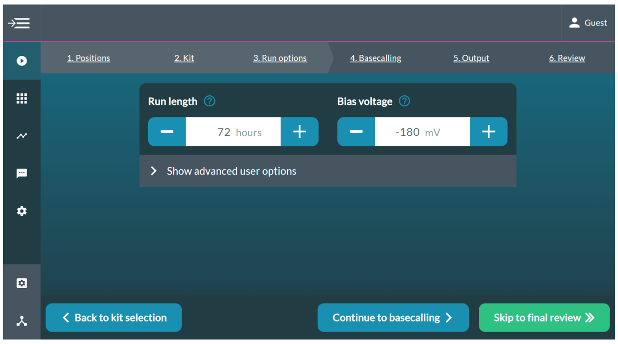


7 **In Run Options, run length and bias voltage can be altered.**

The run options tab provides variables for run time and starting voltage. By default, these will be 72 hours and -180 mV.

Select and type or use the **+** and **—** to increase or decrease settings.

Select **Continue to Basecalling** to proceed to the next section.



TIP

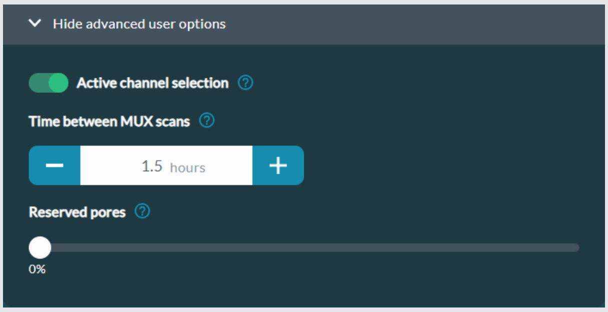
Advanced User Options

Options to turn active channel selection either on or off.

Active channel selection refers to a feature whereby if a pore is in the 'Saturated' or 'Multiple' state, the software instantly switches to a new pore in the group. If a pore is 'Recovering', MinKNOW will attempt to revert the pore back to 'Pore' or 'Sequencing' for ~5 minutes, after which it will select a new pore in the group. This maximises the number of pores sequencing at the start of the experiment.

Time between Mux scans and percentage of reserved pores can be altered to maximise pores for sequencing.

Percentage of reserved pores may also be selected here.



Active channel selection

Time between MUX scans: 1.5 hours

Reserved pores: 0%

8 **Choose basecalling, barcoding and alignment options.**

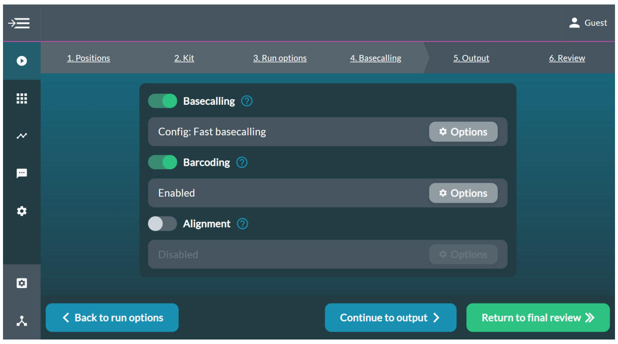
Barcoding options are only available when a barcoding expansion pack has been selected.

If the user does not wish to demultiplex, unselect the check box to disable barcoding.

To use alignment during sequencing, select the check box and upload an alignment reference file as a .fasta or minimap index file.

Note: For barcoding or alignment to be performed during sequencing, basecalling *must* be enabled.

Select **Continue to Output** to proceed.



Basecalling

Config: Fast basecalling

Barcoding

Enabled

Alignment

Disabled

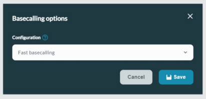
TIP

User options

Use the **Options** button to further customise a run:

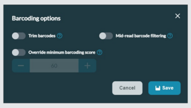
1. Choose basecall model
2. Trim barcodes or alter barcoding score
    - Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
    - Barcoding score can be increased if the user wants to be more confident in barcode detection classification.
3. Enter an alignment reference
    - We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.
    - A reference file must be uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes). Alignment hits from these files are used to populate the alignment graphs.
    - A .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in a chromosome). Alignment hits from .bed files will be highlighted in the sequencing .txt file generated in the data folder.

1.

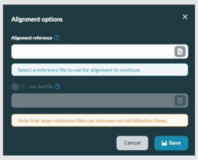


Configuration: Fast basecalling

2.



3.



Select a reference file to use for alignment to continue.

Note that large reference files can increase run initialisation times.

Each barcode demultiplexed by the basecaller will have its own folder and can be processed separately:

```
/data/fastq_pass/barcode[01-XX]
/data/fastq-fail/barcode[01-XX]
```

Note: Barcoding and alignment options are only available when basecalling is enabled.

For more information regarding barcoding, demultiplexing, and alignment, please refer to the Guppy protocol.

Basecall models

Basecalling models can be selected at two stages in the MinKNOW software:

- **Setting up an experiment in MinKNOW** - the instructions for selecting a model can be found in the section 'Starting a sequencing run' of this protocol.
- **Using post-run basecalling in MinKNOW** - the instructions for how to basecall data once an experiment has finished can be found in the section 'Post-run analysis' of this protocol.

Guppy, the basecaller in MinKNOW, provides multiple models for basecalling nanopore data.

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

1. Fast basecalling - This model is able to keep up with a high-throughput sequencing experiment on a MinION Mk1B, MinION Mk1C, GridION or PromethION. This delivers up to 95.8% single-molecule accuracy.

2. High-accuracy basecalling (HAC) - This provides a higher (up to 97.8%) single-molecule accuracy than the Fast model. It is currently 5-8 times more computationally-intensive than the Fast model, so users should ensure their data transfer and device utilisation is scaled appropriately for this.

3. Super Accurate basecalling (SUP) - The Super accurate model has an even higher single-molecule accuracy (up to 98.3%), and is ~3 times more intensive than the HAC model.

4. Modified basecalling for 5mC - This model is provided in MinKNOW and allows the user to basecall certain modified bases (currently 5mC). To understand how the basecaller assigns the basecalled modifications, please read the Stand-alone Guppy protocol, under Output - Modified basecalling.

4. No basecalling

Note: GPU devices will basecall in near real-time; CPU devices, like a standard laptop for MinION Mk1B, will not maintain real-time basecalling.

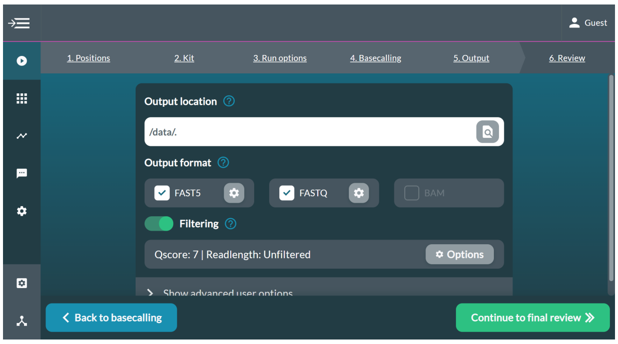
9 **Select the output data location, format and filtering options.**

Use the tick boxes to save output data as FAST5, FASTQ and/or BAM files.

An alternative location can be selected to which processed reads will be written out by using **Output Location**.

Filtering options can be used to determine which reads are classed into pass or fail files. These options may also be used to determine which predefined reads, read lengths and Qscore during basecalling can be split out in some live graphs.

Select **Continue to final review** to view a summary of all run settings.



Output location: /data/.

Output format:
☑ FAST5
☑ FASTQ
☐ BAM

Filtering: on

Qscore: 7 | Readlength: Unfiltered

The default number of reads per FASTQ cannot be changed in the MinKNOW GUI on MinION Mk1C, unlike MinKNOW on other platforms. The number of reads is different according to which flow cell running:

Flongle Flow Cell: 1000
MinION Flow Cell: 4000

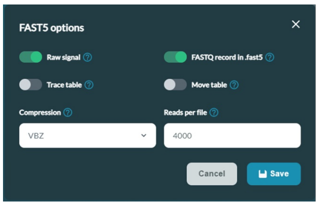
Further options:

Select **Options** in the Output Format panel of the Output tab.

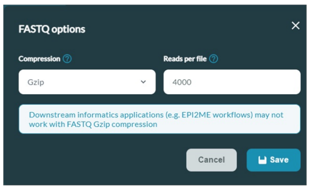
- FAST5: The number of basecalls that MinKNOW will write in a single file.
  - By default, 4000 reads are written out per file
  - The files can be compressed with zlib or VBZ (note: VBZ compression will require a VBZ plugin for compatibility with existing tools. This is optimised for nanopore data, with improved compression over zlib)
- FASTQ: The number of files that MinKNOW will write in a single folder.
  - By default, 4000 files are written into a single folder
  - The files can be uncompressed, or compressed with Gzip to ~55% of original size
- BAM: Files containing aligned reads (one BAM file per input FASTQ file).

Users can change reads per file:

- Fewer reads per file: reads become more quickly available. However, too few reads per file means MinKNOW may not keep up with writing out files in real-time.
- More reads per file: reduces the number of files especially for amplicon/cDNA experiments that produce a large number of reads. Some downstream analysis tools may have an upper limit on the uploaded file size.



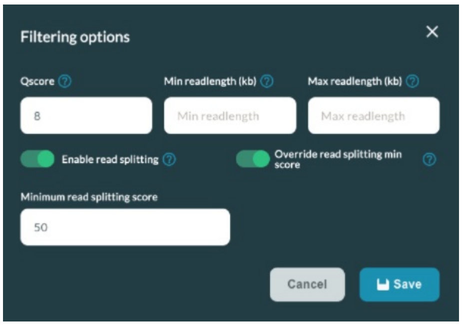
Raw signal: on
FASTQ record in .fast5: on
Trace table: off
Move table: off
Compression: VBZ
Reads per file: 4000



Compression: Gzip
Reads per file: 4000

Downstream informatics applications (e.g. EPI2ME workflows) may not work with FASTQ Gzip compression

Filtering options allows the user to alter Qscore and mid and max read length. Users can also enable read splitting which is a tool used to classify concatermerised reads as "informatic chimeras" for our Kit 12 chemistry. For more information, please see the Kit 12 device and informatics info sheet .



Qscore: 8
Min readlength (kb): Min readlength
Max readlength (kb): Max readlength
Enable read splitting: on
Override read splitting min score: on
Minimum read splitting score: 50

Chosen options can be saved by selecting **Save** for future use.

TIP

Advanced User Options

Option to turn bulk file saving either on or off.

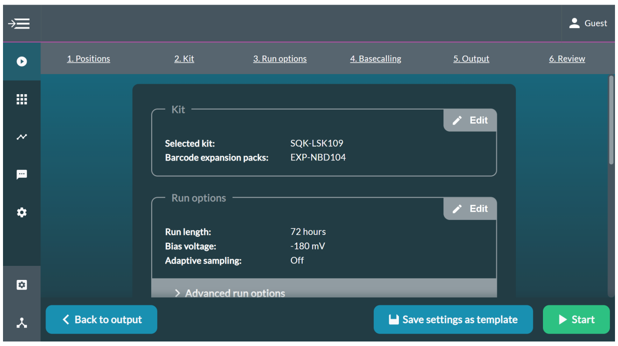
Note: This will result in much larger file sizes due to additional information about the run, which is used for debugging.

10 Select 'Start' to run the experiment.

The **Review** page is an overview of all run options selected.

Edits can be made by selecting the **Edit** button.

Select **Advanced run options** to view the extra options selected.



Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run length: 72 hours
Bias voltage: -180 mV
Adaptive sampling: Off

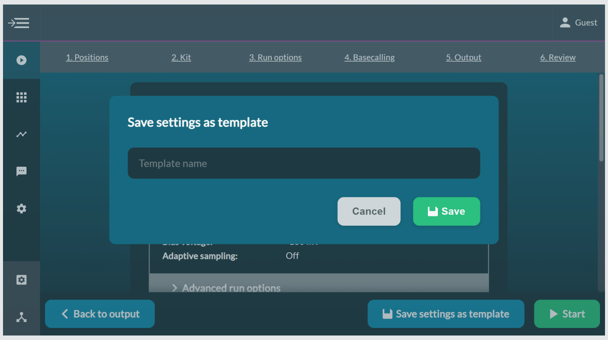
TIP

Save settings

Save settings can be reloaded in following runs as outlined in the **Saved and default settings** tip.

Select **Save settings** once all settings have been chosen.

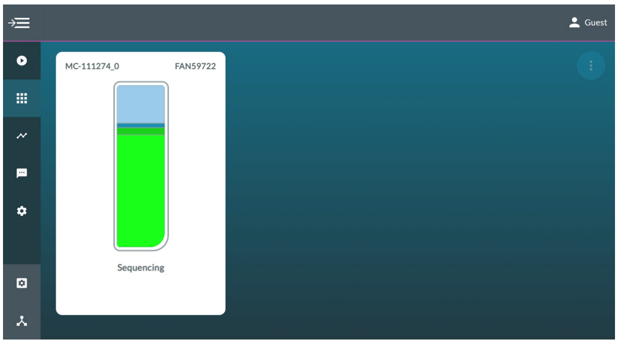
Fill in settings name and select **Save**.



11 Users will be automatically navigated to the Sequencing Overview when sequencing starts.

From here, users can see a progression bar below the flow cell to show the progression of the sequencing script.

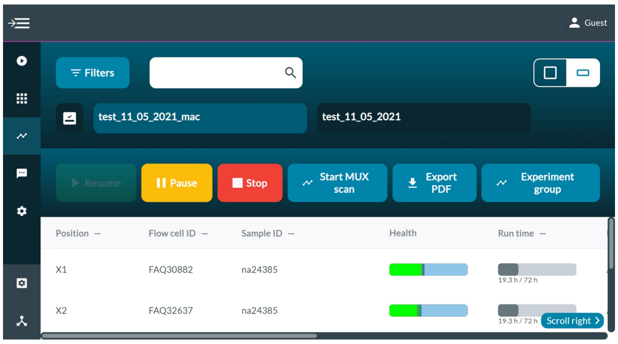
Flow cell health will be displayed after the first MUX scan.



12 Select the flow cell to open the quick view and check the number of active pores. The first Mux scan should report a similar number of active pores (within 10-15%) to that reported in the flow cell check.

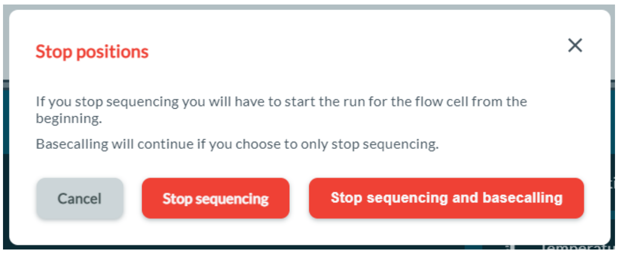
- If there is a significant reduction in active pores in the first Mux scan, restart MinKNOW.
- If the numbers are still significantly different, close down the host computer and reboot.
- When the numbers are similar to those reported at the end of the flow cell check, restart the experiment. There is no need to load any additional library after the restart.

13 To stop the experiment, select 'Stop' on the experiments page.



Position	Flow cell ID	Sample ID	Health	Run time
X1	FAQ30882	na24385		19.3 h / 72 h
X2	FAQ32637	na24385		19.3 h / 72 h

14 In the new window, select either 'Stop sequencing' or 'Stop sequencing and basecalling'.



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

Note: If you select 'Stop sequencing' any catch-up basecalling will still occur. A progress bar will appear on associated flow cell on the Sequencing Overview tab.

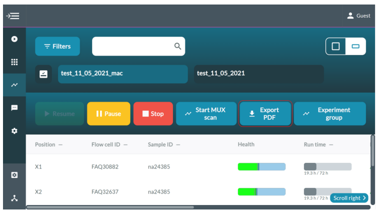
15 **Once sequencing is complete, generate a run report.**

A PDF report containing information about the sequencing run and performance graphs can be generated by right clicking (or press and hold on MinION Mk1C) a run and selecting **Export PDF**.

Note: PDF reports are automatically generated for MinION Mk1C, GridION and PromethION experiments. For other platforms (e.g. MinION Mk1B running on a laptop), the PDF reports need to be manually generated using the **Export PDF** option.

A duty time CSV file is also generated for every run.

The report PDF and CSV files are saved to the same folder as the .fast5 and .fastq read files e.g. :\data\experiment\sample_ID\ for MinION Mk1B.

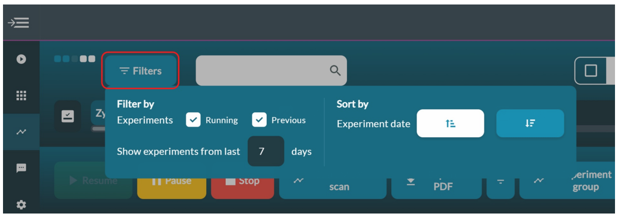


Position	Flow cell ID	Sample ID	Health	Run time
X1	FAQ30882	na24385		19.3 h / 72 h
X2	FAQ32637	na24385		19.3 h / 72 h


Select runs to export PDFs

MC-110168_0 - no_sample - 11/05/2021, 14:30:32

To access previous sequencing runs and view them on the UI, click 'Filters' and select how many days previous to show past experiments.



Filter by
Experiments ☑ Running ☑ Previous
Show experiments from last 7 days
Sort by
Experiment date

END OF STEP

MinKNOW fallback folder

If writing out reads to your specified location fails (e.g. if the disk becomes full, or the connection is disrupted), the experiment will continue, and the reads will be written into a fallback folder:

Windows
.fast5 files: C:\data\reads\fallback (with the standard run data name sample ID as a folder)
FASTQ files: C:\data\reads\fastq_fallback (with the standard run data name sample ID as a folder)

Mac OS X:
.fast5 files: /Library/MinKNOW/data/fallback
FASTQ files: /Library/MinKNOW/data/fastq_fallback

Linux:
.fast5 files: /var/lib/MinKNOW/data/fallback
FASTQ files: /var/lib/MinKNOW/data/fastq_fallback

Starting a sequencing run on GridION

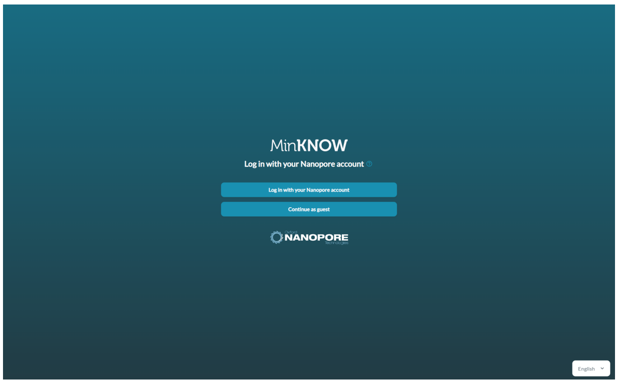
1 **Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI.**

2 **We recommend users to log into the MinKNOW software using their Community credentials.**

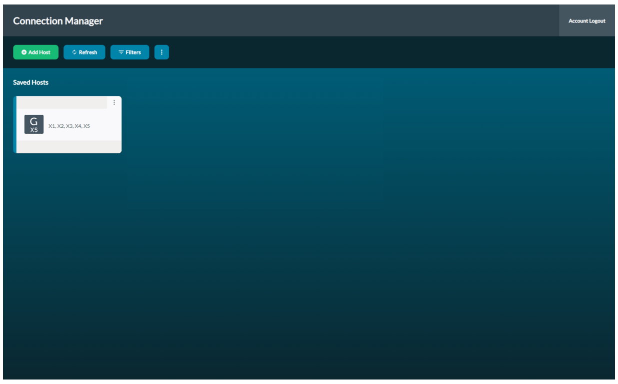
If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

To log in, you must be connected to the internet.

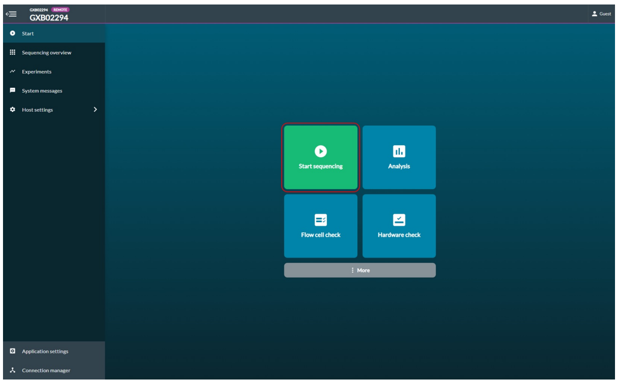
From here, users may also alter language in the user interface in the bottom right corner.



3 **Select the sequencing device connected to the computer.**



4 **Select the 'Start Sequencing' option on the Start homepage to choose the running parameters for your experiment.**



Optional Action
Upload a sample sheet from the start page when running multiple sample names and their corresponding flow cell positions from a CSV file rather than manually.

Please see Sample sheet upload for instructions.

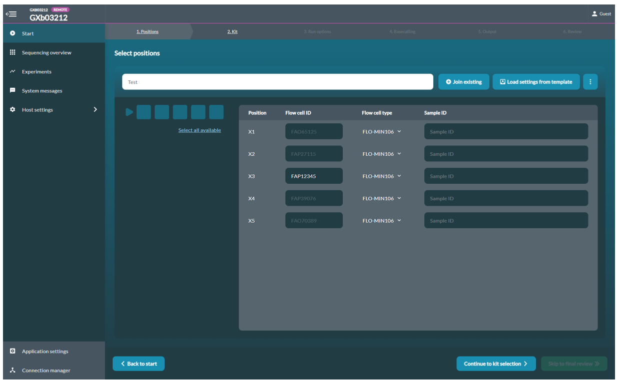
5 **Type in the experiment name, sample ID and choose flow cell type from the drop down menu.**

Ensure the experiment name and sample ID does not contain any personally identifiable information.

Use 'Select all available' to select all the connected flow cells or use the diagram above to select specific flow cells for an experiment.

Note: If sample ID is not filled in, there will be no sample ID in the folder structure.

Select **Continue to Kit Selection** to move to the next page.



TIP

Saved and default settings

To use settings saved from previous runs:

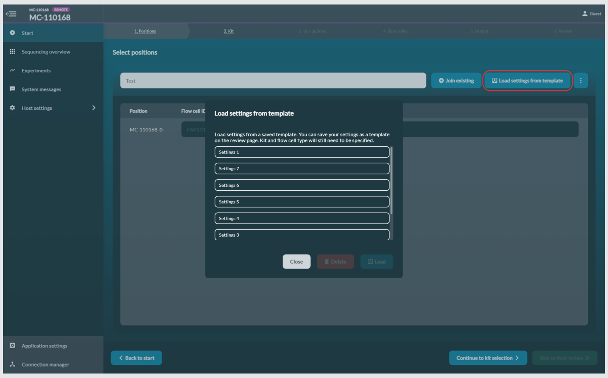
1. Click **Load settings from template**
2. Choose the saved settings to use
3. Select **Load**

Saved settings can be deleted from this window by selecting **Delete**.

Once the saved settings are loaded, the user is able to look through all options and make any changes, if needed, before starting the experiment.

The **Saved Settings** tip further down explains how to save settings.

To restore default run settings, select the three dots and select **Reload Scripts**.



Load settings from a saved template. You can save your settings as a template on the review page. Kit and flow cell type will still need to be specified.

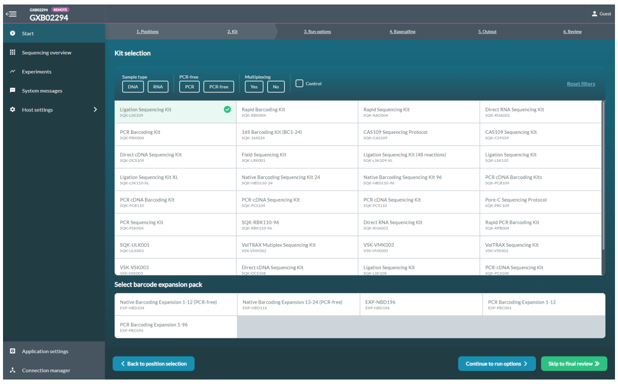
6 **Select the kit used from the Kit selection menu.**

The filter options may be used to find the kit used. For example, if running a Control Experiment, check the **Control** box on the right.
Note: The five most popular kits are displayed in the top rows.

Demultiplexing is referred to as barcoding in this section. If you have used a barcoding kit/expansion pack for your library preparation, the demultiplexing option in MinKNOW can be used to split your reads by barcode without having to use command line-tools. Demultiplexing places reads into barcode-specific folders.

Once a kit is selected, choose the appropriate option from **Select Barcode Expansion Pack** options if a barcode expansion pack has been used.
Note: Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear.

Select **Continue to Run Options** to choose run parameters.



Kit selection: Ligation Sequencing Kit SQK-LSK109 ✓

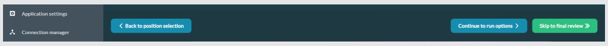
TIP

Skip to final review

To skip to the end of Start Sequencing, select **Skip to final review**.

The user is also able to return to any previous pages by using the **Back to** button.

We recommend users keep the default settings.



7 **In Run Options, run length and bias voltage can be altered and adaptive sampling enabled.**

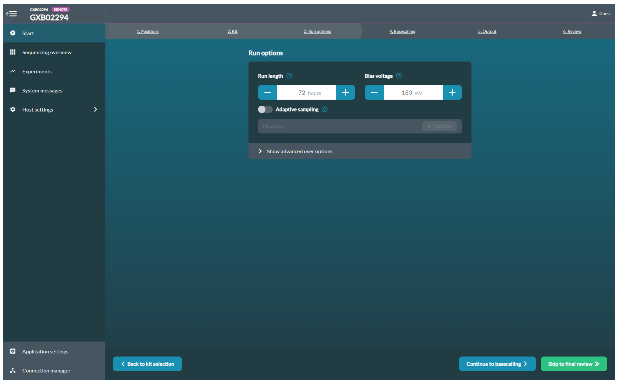
The run options tab provides variables for run time and starting voltage. By default, these will be 72 hours and -180 mV.

Select and type or use the **+** and **—** to increase or decrease settings.

Tick to enable adaptive sampling; further options will then appear. Under **Alignment reference**, select `/data/nanopore_adaptive_seq_cosmic-slop-5000_k-15_w-10.mmi`

Select **Continue to Basecalling** to proceed to the next section. If you have enabled adaptive sampling, the basecalling options will be unavailable. For the MinION Mk1C adaptive sampling beta release, regular basecalling of entire reads is disabled during the sequencing experiment, and basecalling should be initiated manually post-run.

Note: We recommend using alignment on bacterial-sized genomes.



Run options
Run length: 72 hours
Bias voltage: -180 mV
Adaptive sampling: Disabled

8 **If adaptive sampling is enabled, add an alignment reference in Options.**

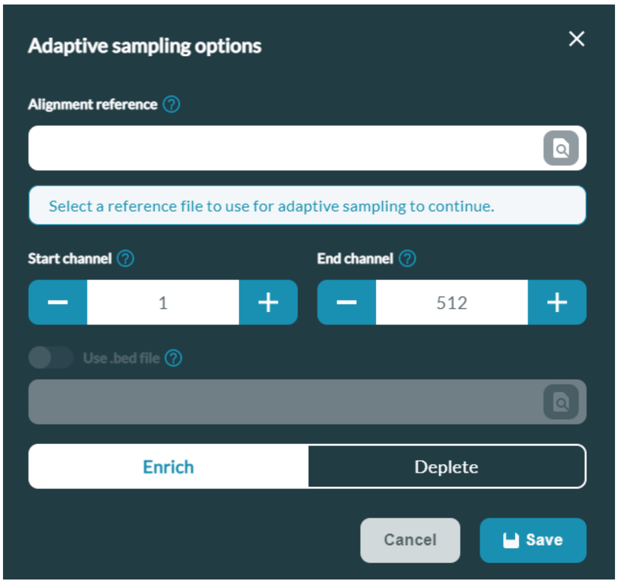
An alignment reference must be a FASTA or Minimap2 file.

The specified channel range may also be altered by typing or using the **+** or **–**.

Add a .bed file to subset the alignment reference.

Select **Enrich** or **Deplete** to enrich or deplete the alignment reference.

For more information, please refer to the Adaptive sampling info sheet.



Adaptive sampling options

Select a reference file to use for adaptive sampling to continue.

Start channel: 1

End channel: 512

TIP

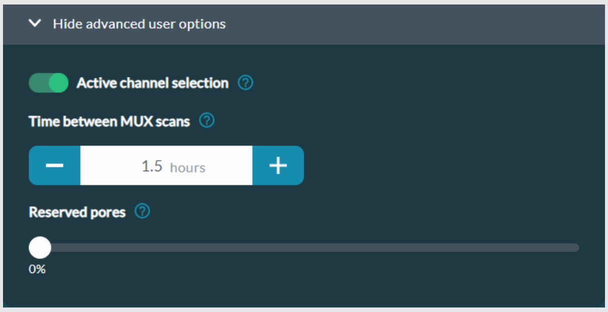
Advanced User Options

Options to turn active channel selection either on or off.

Active channel selection refers to a feature whereby if a pore is in the 'Saturated' or 'Multiple' state, the software instantly switches to a new pore in the group. If a pore is 'Recovering', MinKNOW will attempt to revert the pore back to 'Pore' or 'Sequencing' for ~5 minutes, after which it will select a new pore in the group. This maximises the number of pores sequencing at the start of the experiment.

Time between Mux scans and percentage of reserved pores can be altered to maximise pores for sequencing.

Percentage of reserved pores may also be selected here.



Active channel selection: on

Time between MUX scans: 1.5 hours

Reserved pores: 0%

9 Choose basecalling, barcoding and alignment options.

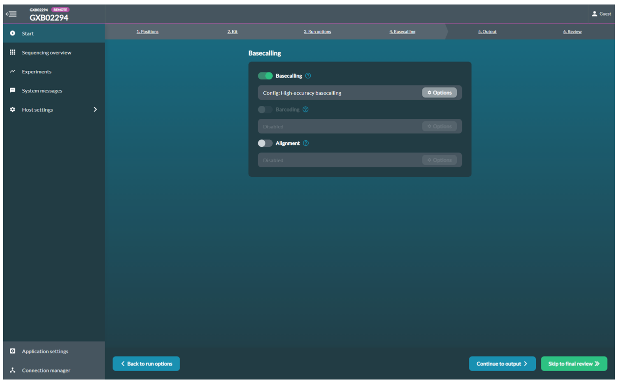
Barcoding options are only available when a barcoding expansion pack has been selected.

If the user does not wish to demultiplex, unselect the toggle to disable barcoding.

To use alignment during sequencing, select the toggle and upload an alignment reference file as a .fasta or minimap index file.

Note: For barcoding or alignment to be performed during sequencing, basecalling *must* be enabled.

Select **Continue to Output** to proceed.



Config: High-accuracy basecalling

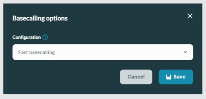
TIP

User options

Use the **Options** button to further customise a run:

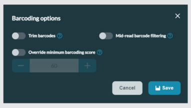
1. Choose basecall model
2. Trim barcodes or alter barcoding score
    - Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
    - Barcoding score can be increased if the user wants to be more confident in barcode detection classification.
3. Enter an alignment reference
    - We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.
    - A reference file must be uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes). Alignment hits from these files are used to populate the alignment graphs.
    - A .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in a chromosome). Alignment hits from .bed files will be highlighted in the sequencing .txt file generated in the data folder.

1.



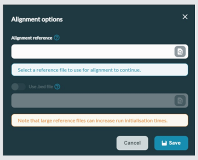
Configuration: Fast basecalling

2.



60

3.



Select a reference file to use for alignment to continue.

Note that large reference files can increase run initialisation times.

Each barcode demultiplexed by the basecaller will have its own folder and can be processed separately:

```
/data/fastq_pass/barcode[01-XX]
/data/fastq-fail/barcode[01-XX]
```

Note: Barcoding and alignment options are only available when basecalling is enabled.

For more information regarding barcoding, demultiplexing, and alignment, please refer to the Guppy protocol.

Basecall models

Basecalling models can be selected at two stages in the MinKNOW software:

- **Setting up an experiment in MinKNOW** - the instructions for selecting a model can be found in the section 'Starting a sequencing run' of this protocol.
- **Using post-run basecalling in MinKNOW** - the instructions for how to basecall data once an experiment has finished can be found in the section 'Post-run analysis' of this protocol.

Guppy, the basecaller in MinKNOW, provides multiple models for basecalling nanopore data.

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

1. Fast basecalling - This model is able to keep up with a high-throughput sequencing experiment on a MinION Mk1B, MinION Mk1C, GridION or PromethION. This delivers up to 95.8% single-molecule accuracy.

2. High-accuracy basecalling (HAC) - This provides a higher (up to 97.8%) single-molecule accuracy than the Fast model. It is currently 5-8 times more computationally-intensive than the Fast model, so users should ensure their data transfer and device utilisation is scaled appropriately for this.

3. Super Accurate basecalling (SUP) - The Super accurate model has an even higher single-molecule accuracy (up to 98.3%), and is ~3 times more intensive than the HAC model.

4. Modified basecalling for 5mC - This model is provided in MinKNOW and allows the user to basecall certain modified bases (currently 5mC). To understand how the basecaller assigns the basecalled modifications, please read the Stand-alone Guppy protocol, under Output - Modified basecalling.

4. No basecalling

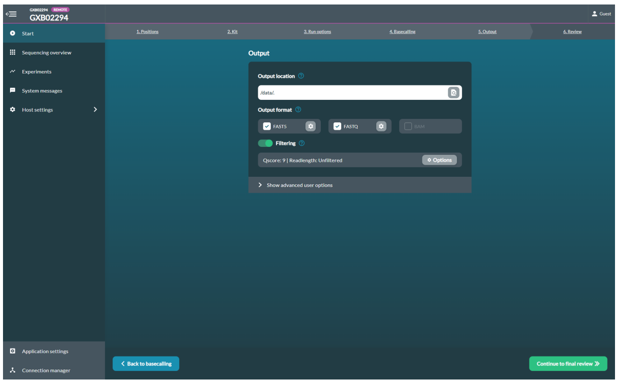
Note: GPU devices will basecall in near real-time; CPU devices, like a standard laptop for MinION Mk1B, will not maintain real-time basecalling.

10 Select the output data location, format and filtering options.

Use the checkboxes to save output data as FAST5, FASTQ and/or BAM files.
An alternative location can be selected to which processed reads will be written out by using **Output Location**.

Filtering options can be used to determine which reads are classed into pass or fail files. These options may also be used to determine which predefined reads, read lengths and Qscore during basecalling can be split out in some live graphs.

Select **Continue to final review** to proceed.



Output location: /data/.

Output format: ☑ FAST5 ☑ FASTQ ☐ BAM

Filtering: Qscore: 9 | Readlength: Unfiltered

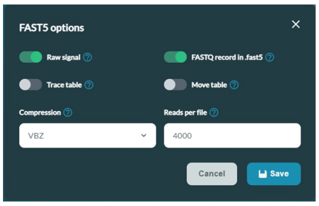
Further options:

Select **Options** in the Output Format panel of the Output tab.

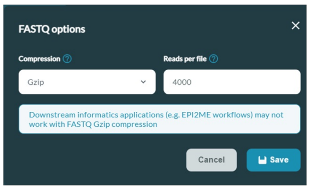
- FAST5: The number of basecalls that MinKNOW will write in a single file.
  - By default, 4000 reads are written out per file
  - The files can be compressed with zlib or VBZ (note: VBZ compression will require a VBZ plugin for compatibility with existing tools. This is optimised for nanopore data, with improved compression over zlib)
- FASTQ: The number of files that MinKNOW will write in a single folder.
  - By default, 4000 files are written into a single folder
  - The files can be uncompressed, or compressed with Gzip to ~55% of original size
- BAM: Files containing aligned reads (one BAM file per input FASTQ file).

Users can change reads per file:

- Fewer reads per file: reads become more quickly available. However, too few reads per file means MinKNOW may not keep up with writing out files in real-time.
- More reads per file: reduces the number of files especially for amplicon/cDNA experiments that produce a large number of reads. Some downstream analysis tools may have an upper limit on the uploaded file size.



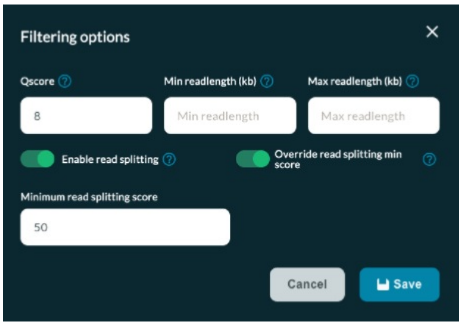
Raw signal: on
FASTQ record in .fast5: on
Trace table: off
Move table: off
Compression: VBZ
Reads per file: 4000



Compression: Gzip
Reads per file: 4000

Downstream informatics applications (e.g. EPI2ME workflows) may not work with FASTQ Gzip compression

Filtering options allows the user to alter Qscore and mid and max read length. Users can also enable read splitting which is a tool used to classify concatermerised reads as "informatic chimeras" for our Kit 12 chemistry. For more information, please see the Kit 12 device and informatics info sheet .



Qscore: 8
Min readlength (kb): Min readlength
Max readlength (kb): Max readlength
Enable read splitting
Override read splitting min score
Minimum read splitting score: 50

Chosen options can be saved by selecting **Save** for future use.

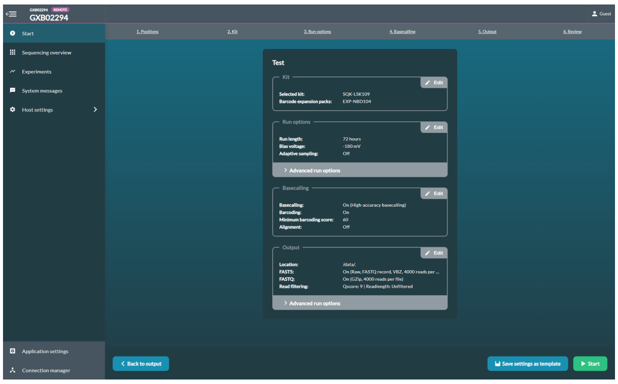
> **TIP**
>
> **Advanced User Options**
>
> Option to turn bulk file saving either on or off.
>
> **Note:** This will result in much larger file sizes due to additional information about the run, which is used for debugging.
>
> 
>
>
> Output bulk file

11 **Select 'Start' to run the experiment.**

The **Review** page is an overview of all run options selected.

Edits can be made by selecting the **Edit** button.

Select **Advanced run options** to view the extra options selected.



Test

Kit
Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options
Run length: 72 hours
Bias voltage: -180 mV
Adaptive sampling: Off
Advanced run options

Basecalling
Basecalling: On (High-accuracy basecalling)
Barcoding: On
Minimum barcoding score: 60
Alignment: Off

Output
Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered
Advanced run options

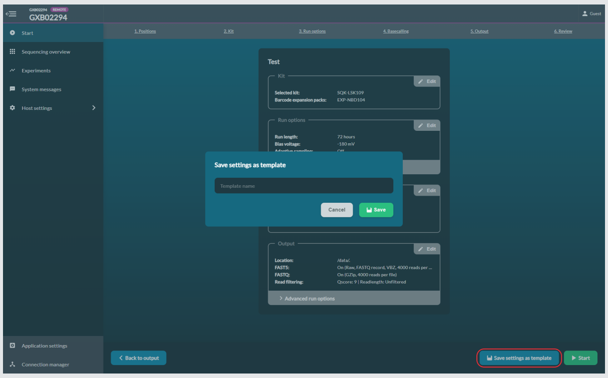
TIP

Save settings

Save settings to reload in following runs as outlined in the **Saved and default settings** tip.

Select **Save settings as template** once all settings have been chosen.

Fill in settings name and select **Save**.



Test

Kit
Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options
Run length: 72 hours
Bias voltage: -180 mV

Output
Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

12 **Users will be automatically navigated to the Sequencing Overview when sequencing starts.**

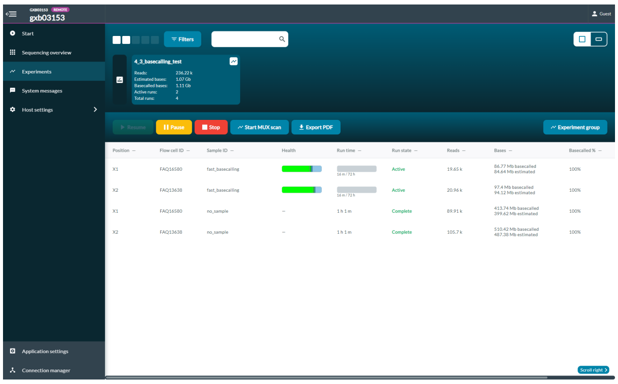
From here, users can see a progression bar below each flow cell to show progression of the sequencing script.

Flow cell health will be displayed after the first Mux scan.

13 **Select the flow cell to open the quick view and check the number of active pores. The first Mux scan should report a similar number of active pores (within 10-15%) to that reported in the flow cell check.**

- If there is a significant reduction in active pores in the first Mux scan, restart MinKNOW.
- If the numbers are still significantly different, close down the host computer and reboot.
- When the numbers are similar to those reported at the end of the flow cell check, restart the experiment. There is no need to load any additional library after the restart.

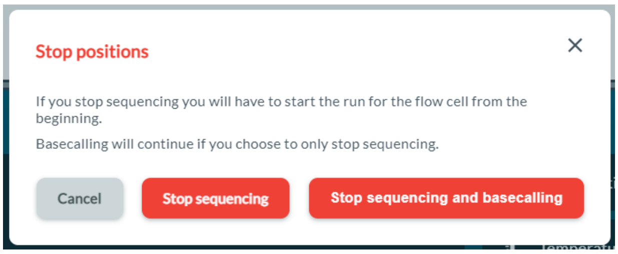
14 **To stop the experiment, select 'Stop' on the experiments page.**



4_3_basecalling_test
Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%
X2	FAQ13638	fast_basecalling		16 m / 72 h	Active	20.96 k	97.4 Mb basecalled 94.12 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

15 In the new window, select either 'Stop sequencing' or 'Stop sequencing and basecalling'.



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

Note: If you select 'Stop sequencing' any catch-up basecalling will still occur. A progress bar will appear on associated flow cell on the Sequencing Overview tab.

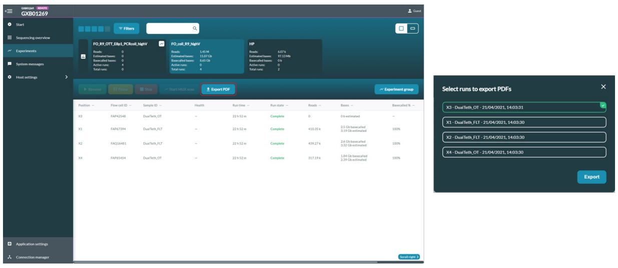
16 Once sequencing is complete, generate a run report.

A PDF report containing information about the sequencing run and performance graphs can be generated by clicking **Export PDF**.

PDF reports are automatically generated for MinION Mk1C, GridION and PromethION experiments. For other platforms (e.g. MinION Mk1B running on a laptop), the PDF reports need to be manually generated. Click **Export PDF** and select which experiment to export PDF.

A duty time CSV file is also generated for every run.

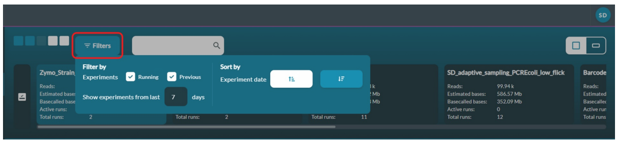
The report PDF and CSV files are saved to the same folder as the .fast5 and .fastq read files e.g. :\data\experiment\sample_ID\ for MinION Mk1B.



Select runs to export PDFs

- X3 - DualTeth_OT - 21/04/2021, 14:03:31
- X1 - DualTeth_FLT - 21/04/2021, 14:03:30
- X2 - DualTeth_FLT - 21/04/2021, 14:03:30
- X4 - DualTeth_OT - 21/04/2021, 14:03:30

To access previous sequencing runs and view them on the UI, click 'Filters' and select how many days previous to show past experiments.



Filter by
Experiments ☑ Running ☑ Previous
Show experiments from last 7 days

Sort by
Experiment date

SD_adaptive_sampling_PCREcoli_low_flick
Reads: 99.94 k
Estimated bases: 586.57 Mb
Basecalled bases: 352.09 Mb
Active runs: 0
Total runs: 12

END OF STEP

MinKNOW fallback folder

If writing out reads to your specified location fails (e.g. if the disk becomes full, or the connection is disrupted), the experiment will continue, and the reads will be written into a fallback folder:

Windows
.fast5 files: C:\data\reads\fallback (with the standard run data name sample ID as a folder)
FASTQ files: C:\data\reads\fastq_fallback (with the standard run data name sample ID as a folder)

Mac OS X:
.fast5 files: /Library/MinKNOW/data/fallback
FASTQ files: /Library/MinKNOW/data/fastq_fallback

Linux:
.fast5 files: /var/lib/MinKNOW/data/fallback
FASTQ files: /var/lib/MinKNOW/data/fastq_fallback

Starting a sequencing run on PromethION

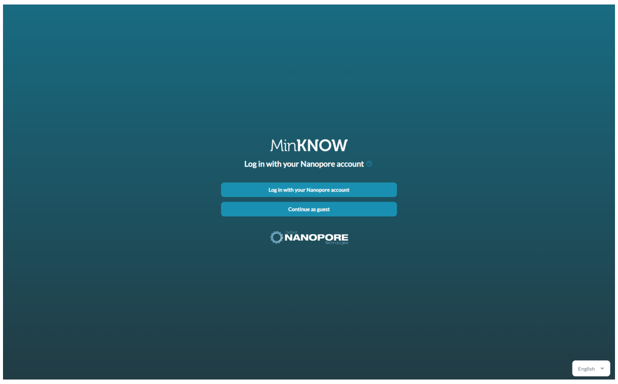
1 **Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI.**

2 **We recommend users to log into the MinKNOW software using their Community credentials.**

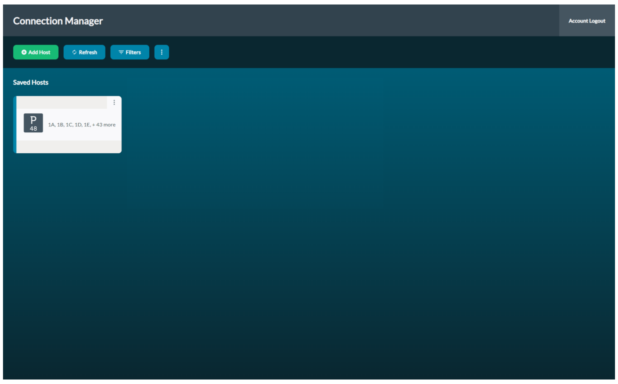
If you experience login issues, please visit the Community Support channel (https://community.nanoporetech.com/support) and use **Continue as guest** for temporary use.

To log in, you must be connected to the internet.

From here, users may also alter language in the user interface in the bottom right corner.



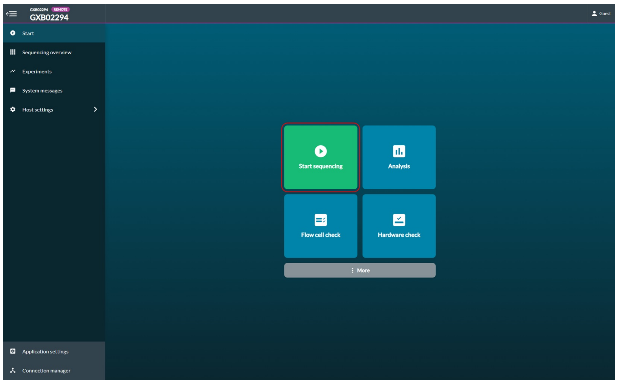
3 **Select the sequencing device connected to the computer.**



IMPORTANT

After taking flow cells out of the fridge, wait 20 minutes before inserting the flow cell into the PromethION for the flow cell to come to room temperature. Condensation can form on the flow cell in humid environments. Inspect the gold connector pins on the top and underside of the flow cell for condensation and wipe off with a Kimwipe if any is observed. Ensure the heat pad (black pad) is present on the ASIC.

4 **Select the 'Start Sequencing' option on the Start homepage to choose the running parameters for your experiment.**



Optional Action
Upload a sample sheet from the start page when running multiple sample names and their corresponding flow cell positions from a CSV file rather than manually.

Please see Sample sheet upload for instructions.

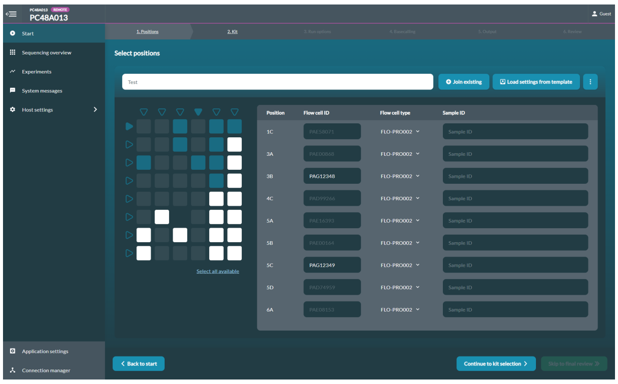
5 **Type in the experiment name, sample ID and choose flow cell type from the drop down menu.**

Select **Continue to Kit Selection** to move to the next page.

Ensure the experiment name and sample ID does not contain any personally identifiable information.

Use 'Select all available' to select all the connected flow cells or use the diagram above to select specific flow cells for an experiment.

Note: If sample ID is not filled in, there will be no sample ID in the folder structure.



Test

Position	Flow cell ID	Flow cell type	Sample ID
1C	PAE58071	FLO-PRO002	Sample ID
3A	PAE00868	FLO-PRO002	Sample ID
3B	PAG12348	FLO-PRO002	Sample ID
4C	PAD99266	FLO-PRO002	Sample ID
5A	PAE16393	FLO-PRO002	Sample ID
5B	PAE00164	FLO-PRO002	Sample ID
5C	PAG12349	FLO-PRO002	Sample ID
5D	PAD74959	FLO-PRO002	Sample ID
6A	PAE08153	FLO-PRO002	Sample ID

TIP

Saved and default settings

To use settings saved from previous runs:

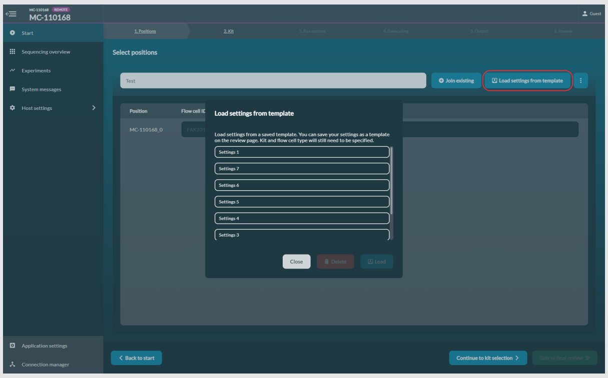
1. Click **Load settings from template**
2. Choose the saved settings to use
3. Select **Load**

Saved settings can be deleted from this window by selecting **Delete**.

Once the saved settings are loaded, the user is able to look through all options and make any changes, if needed, before starting the experiment.

The **Saved Settings** tip further down explains how to save settings.

To restore default run settings, select the three dots and select **Reload Scripts**.



Load settings from template

Load settings from a saved template. You can save your settings as a template on the review page. Kit and flow cell type will still need to be specified.

- Settings 1
- Settings 7
- Settings 6
- Settings 5
- Settings 4
- Settings 3

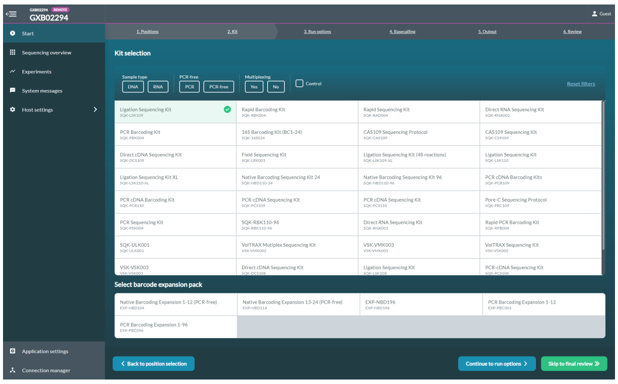
6 **Select the kit used from the Kit selection menu.**

The filter options may be used to find the kit used. For example, if running a Control Experiment, check the **Control** box on the right.
Note: The five most popular kits are displayed in the top rows.

Demultiplexing is referred to as barcoding in this section. If you have used a barcoding kit/expansion pack for your library preparation, the demultiplexing option in MinKNOW can be used to split your reads by barcode without having to use command line-tools. Demultiplexing places reads into barcode-specific folders.

Once a kit is selected, choose the appropriate option from **Select Barcode Expansion Pack** options if a barcode expansion pack has been used.
Note: Two or more barcoding expansion packs can be selected. If a selected kit is not compatible with any barcode expansion pack, barcode options will not appear.

Select **Continue to Run Options** to choose run parameters.



Kit selection

Ligation Sequencing Kit ✓ SQK-LSK109	Rapid Barcoding Kit SQK-RBK004	Rapid Sequencing Kit SQK-RAD004	Direct RNA Sequencing Kit SQK-RNA002
PCR Barcoding Kit SQK-PBK004	16S Barcoding Kit (BC1-24) SQK-16S024	CAS109 Sequencing Protocol SQK-CAS109	CAS109 Sequencing Kit SQK-CS9109
Direct cDNA Sequencing Kit SQK-DCS109	Field Sequencing Kit SQK-LRK001	Ligation Sequencing Kit (48 reactions) SQK-LSK109-XL	Ligation Sequencing Kit SQK-LSK110
Ligation Sequencing Kit XL SQK-LSK110-XL	Native Barcoding Sequencing Kit 24 SQK-NBD110-24	Native Barcoding Sequencing Kit 96 SQK-NBD110-96	PCR cDNA Barcoding Kits SQK-PCB109
PCR cDNA Barcoding Kit SQK-PCB110	PCR-cDNA Sequencing Kit SQK-PCS109	PCR cDNA Sequencing Kit SQK-PCS110	Pore-C Sequencing Protocol SQK-PRC109
PCR Sequencing Kit SQK-PSK004	SQK-RBK110-96 SQK-RBK110-96	Direct RNA Sequencing Kit SQK-RNA003	Rapid PCR Barcoding Kit SQK-RPB004
SQK-ULK001 SQK-ULK001	VolTRAX Mutiplex Sequencing Kit VSK-VMK002	VSK-VMK003 VSK-VMK003	VolTRAX Sequencing Kit VSK-VSK002
VSK-VSK003 VSK-VSK003	Direct cDNA Sequencing Kit SQK-DCS108	Ligation Sequencing Kit SQK-LSK108	PCR-cDNA Sequencing Kit SQK-PCS108

Select barcode expansion pack

Native Barcoding Expansion 1-12 (PCR-free) EXP-NBD104	Native Barcoding Expansion 13-24 (PCR-free) EXP-NBD114	EXP-NBD196 EXP-NBD196	PCR Barcoding Expansion 1-12 EXP-PBC001
PCR Barcoding Expansion 1-96 EXP-PBC096			

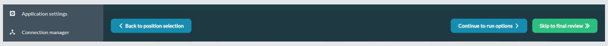
TIP

Skip to final review

To skip to the end of Start Sequencing, select **Skip to final review**.

The user is also able to return to any previous pages by using the **Back to** button.

We recommend users keep the default settings.



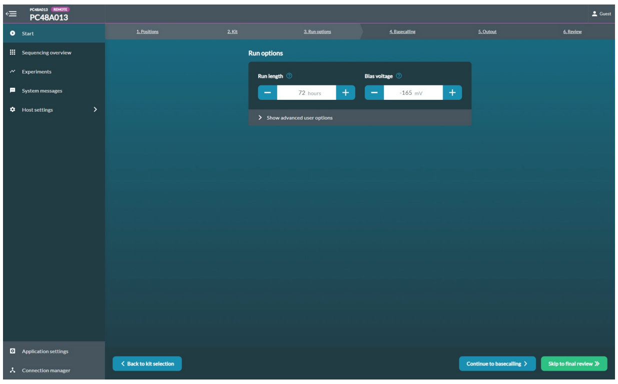
7 **Edit run length and bias voltage in Run Options.**

The run options tab provides variables for run time and starting voltage. By default, these will be 72 hours and -165 mV.

Select and type or use the **+** and **—** to increase or decrease settings.

For more information about adjusting starting voltage, refer to Adjusting the starting potential in this protocol.

Select **Continue to Basecalling** to proceed to the next section.



Run length: 72 hours

Bias voltage: -165 mV

TIP

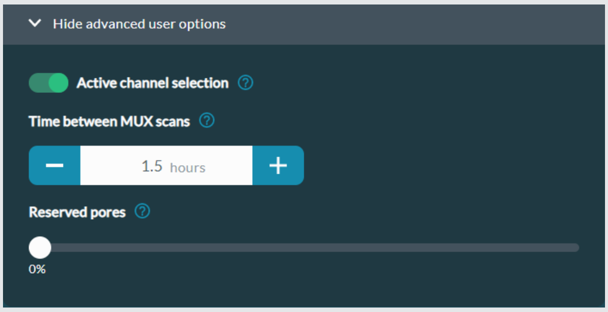
Advanced User Options

Options to turn active channel selection either on or off.

Active channel selection refers to a feature whereby if a pore is in the 'Saturated' or 'Multiple' state, the software instantly switches to a new pore in the group. If a pore is 'Recovering', MinKNOW will attempt to revert the pore back to 'Pore' or 'Sequencing' for ~5 minutes, after which it will select a new pore in the group. This maximises the number of pores sequencing at the start of the experiment.

Time between Mux scans and percentage of reserved pores can be altered to maximise pores for sequencing.

Percentage of reserved pores may also be selected here.



Time between MUX scans: 1.5 hours

Reserved pores: 0%

8 **Choose basecalling, barcoding and alignment options.**

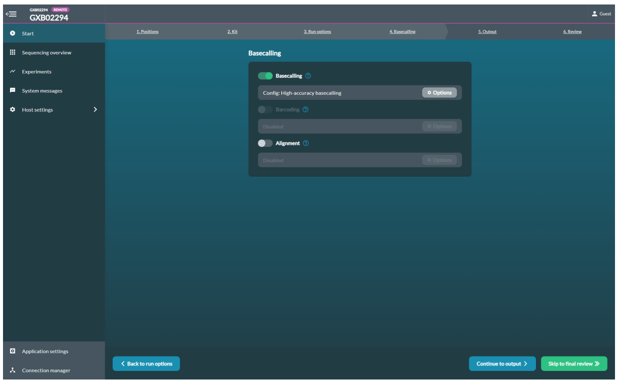
Barcoding options are only available when a barcoding expansion pack has been selected.

If the user does not wish to demultiplex, unselect the toggle to disable barcoding.

To use alignment during sequencing, select the toggle and upload an alignment reference file as a .fasta or minimap index file.

Note: For barcoding or alignment to be performed during sequencing, basecalling *must* be enabled.

Select **Continue to Output** to proceed.



Config: High-accuracy basecalling

Barcoding: Disabled

Alignment: Disabled

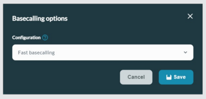
TIP

User options

Use the **Options** button to further customise a run:

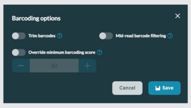
1. Choose basecall model
2. Trim barcodes or alter barcoding score
    - Use the trim barcode options to remove barcodes from demultiplexed reads. Please note, some primer sequences may also be trimmed together with the barcodes.
    - Barcoding score can be increased if the user wants to be more confident in barcode detection classification.
3. Enter an alignment reference
    - We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.
    - A reference file must be uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes). Alignment hits from these files are used to populate the alignment graphs.
    - A .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in a chromosome). Alignment hits from .bed files will be highlighted in the sequencing .txt file generated in the data folder.

1.

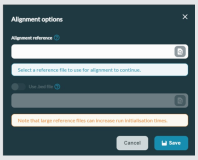


Configuration: Fast basecalling

2.



3.



Select a reference file to use for alignment to continue.

Note that large reference files can increase run initialisation times.

Each barcode demultiplexed by the basecaller will have its own folder and can be processed separately:

```
/data/fastq_pass/barcode[01-XX]
/data/fastq-fail/barcode[01-XX]
```

Note: Barcoding and alignment options are only available when basecalling is enabled.

For more information regarding barcoding, demultiplexing, and alignment, please refer to the Guppy protocol.

Basecall models

Basecalling models can be selected at two stages in the MinKNOW software:

- **Setting up an experiment in MinKNOW** - the instructions for selecting a model can be found in the section 'Starting a sequencing run' of this protocol.
- **Using post-run basecalling in MinKNOW** - the instructions for how to basecall data once an experiment has finished can be found in the section 'Post-run analysis' of this protocol.

Guppy, the basecaller in MinKNOW, provides multiple models for basecalling nanopore data.

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

1. Fast basecalling - This model is able to keep up with a high-throughput sequencing experiment on a MinION Mk1B, MinION Mk1C, GridION or PromethION. This delivers up to 95.8% single-molecule accuracy.

2. High-accuracy basecalling (HAC) - This provides a higher (up to 97.8%) single-molecule accuracy than the Fast model. It is currently 5-8 times more computationally-intensive than the Fast model, so users should ensure their data transfer and device utilisation is scaled appropriately for this.

3. Super Accurate basecalling (SUP) - The Super accurate model has an even higher single-molecule accuracy (up to 98.3%), and is ~3 times more intensive than the HAC model.

4. Modified basecalling for 5mC - This model is provided in MinKNOW and allows the user to basecall certain modified bases (currently 5mC). To understand how the basecaller assigns the basecalled modifications, please read the Stand-alone Guppy protocol, under Output - Modified basecalling.

4. No basecalling

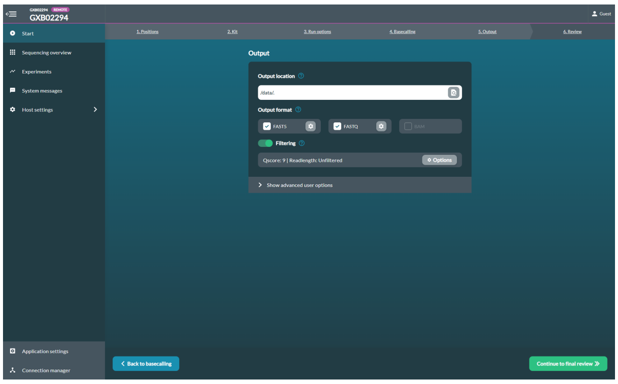
Note: GPU devices will basecall in near real-time; CPU devices, like a standard laptop for MinION Mk1B, will not maintain real-time basecalling.

9 **Select the output data location, format and filtering options.**

Use the checkboxes to save output data as FAST5, FASTQ and/or BAM files.
An alternative location can be selected to which processed reads will be written out by using **Output Location**.

Filtering options can be used to determine which reads are classed into pass or fail files. These options may also be used to determine which predefined reads, read lengths and Qscore during basecalling can be split out in some live graphs.

Select **Continue to final review** to proceed.



Output location: /data/.

Output format: ☑ FAST5 ☑ FASTQ ☐ BAM

Filtering: Qscore: 9 | Readlength: Unfiltered

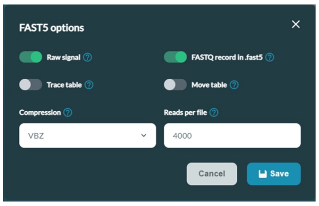
Further options:

Select **Options** in the Output Format panel of the Output tab.

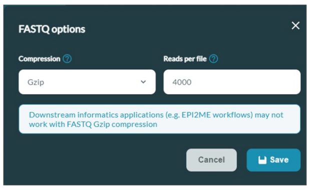
- FAST5: The number of basecalls that MinKNOW will write in a single file.
  - By default, 4000 reads are written out per file
  - The files can be compressed with zlib or VBZ (note: VBZ compression will require a VBZ plugin for compatibility with existing tools. This is optimised for nanopore data, with improved compression over zlib)
- FASTQ: The number of files that MinKNOW will write in a single folder.
  - By default, 4000 files are written into a single folder
  - The files can be uncompressed, or compressed with Gzip to ~55% of original size
- BAM: Files containing aligned reads (one BAM file per input FASTQ file).

Users can change reads per file:

- Fewer reads per file: reads become more quickly available. However, too few reads per file means MinKNOW may not keep up with writing out files in real-time.
- More reads per file: reduces the number of files especially for amplicon/cDNA experiments that produce a large number of reads. Some downstream analysis tools may have an upper limit on the uploaded file size.



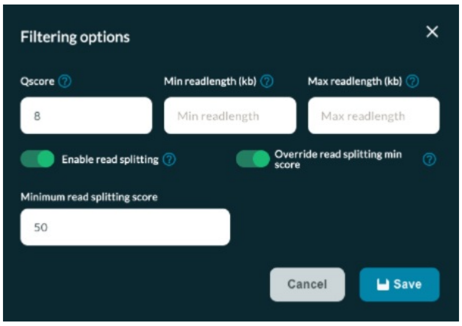
Raw signal: on
FASTQ record in .fast5: on
Trace table: off
Move table: off
Compression: VBZ
Reads per file: 4000



Compression: Gzip
Reads per file: 4000

Downstream informatics applications (e.g. EPI2ME workflows) may not work with FASTQ Gzip compression

Filtering options allows the user to alter Qscore and mid and max read length. Users can also enable read splitting which is a tool used to classify concatermerised reads as "informatic chimeras" for our Kit 12 chemistry. For more information, please see the Kit 12 device and informatics info sheet .



Qscore: 8

Min readlength (kb): Min readlength

Max readlength (kb): Max readlength

Enable read splitting ●

Override read splitting min score ●

Minimum read splitting score: 50

Chosen options can be saved by selecting **Save** for future use.

TIP

Advanced User Options

Option to turn bulk file saving either on or off.

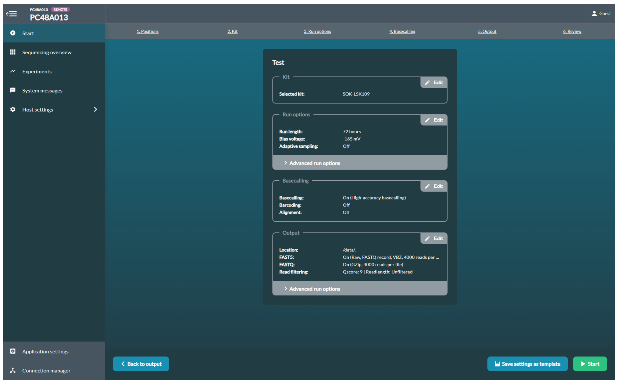
Note: This will result in much larger file sizes due to additional information about the run, which is used for debugging.

10 **Select 'Start' to run the experiment.**

The **Review** page is an overview of all run options selected.

Edits can be made by selecting the **Edit** button.

Select **Advanced run options** to view the extra options selected.



Test

Kit

Selected kit: SQK-LSK109

Run options

Run length: 72 hours
Bias voltage: -165 mV
Adaptive sampling: Off

Basecalling

Basecalling: On (High-accuracy basecalling)
Barcoding: Off
Alignment: Off

Output

Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

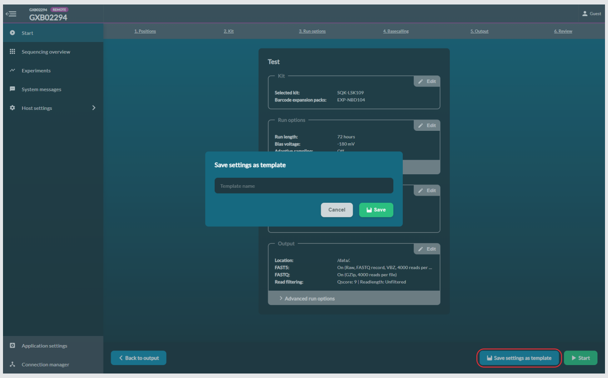
TIP

Save settings

Save settings to reload in following runs as outlined in the **Saved and default settings** tip.

Select **Save settings as template** once all settings have been chosen.

Fill in settings name and select **Save**.



Test

Kit

Selected kit: SQK-LSK109
Barcode expansion packs: EXP-NBD104

Run options

Run length: 72 hours
Bias voltage: -180 mV

Output

Location: /data/.
FAST5: On (Raw, FASTQ record, VBZ, 4000 reads per ...
FASTQ: On (GZip, 4000 reads per file)
Read filtering: Qscore: 9 | Readlength: Unfiltered

11 Users will be automatically navigated to the Sequencing Overview when sequencing starts.

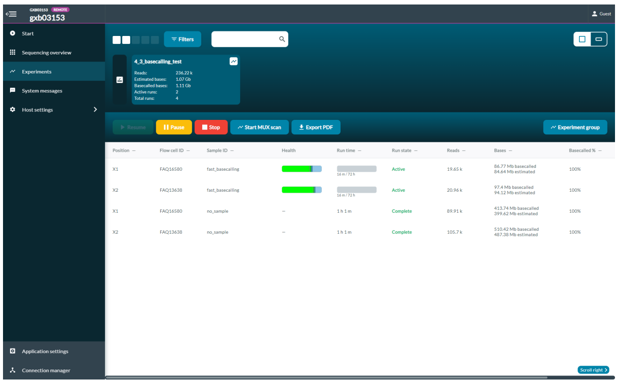
From here, users can see a progression bar below each flow cell to show progression of the sequencing script.

Flow cell health will be displayed after the first Mux scan.

12 Select the flow cell to open the quick view and check the number of active pores. The first Mux scan should report a similar number of active pores (within 10-15%) to that reported in the flow cell check.

- If there is a significant reduction in active pores in the first Mux scan, restart MinKNOW.
- If the numbers are still significantly different, close down the host computer and reboot.
- When the numbers are similar to those reported at the end of the flow cell check, restart the experiment. There is no need to load any additional library after the restart.

13 To stop the experiment, select 'Stop' on the experiments page.

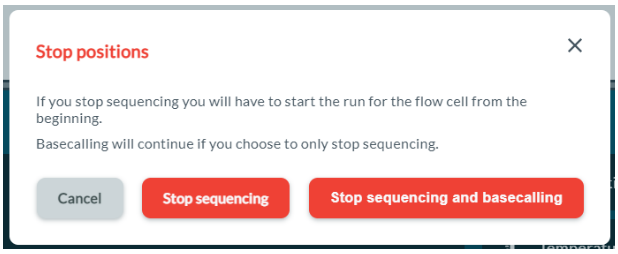


4_3_basecalling_test

Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%
X2	FAQ13638	fast_basecalling		16 m / 72 h	Active	20.96 k	97.4 Mb basecalled 94.12 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

14 In the new window, select either 'Stop sequencing' or 'Stop sequencing and basecalling'.



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

Note: If you select 'Stop sequencing' any catch-up basecalling will still occur. A progress bar will appear on associated flow cell on the Sequencing Overview tab.

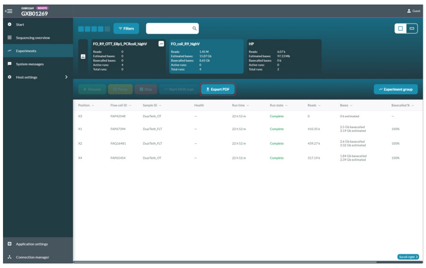
15 Once sequencing is complete, generate a run report.

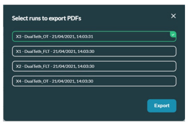
A PDF report containing information about the sequencing run and performance graphs can be generated by clicking **Export PDF**.

PDF reports are automatically generated for MinION Mk1C, GridION and PromethION experiments. For other platforms (e.g. MinION Mk1B running on a laptop), the PDF reports need to be manually generated. Click **Export PDF** and select which experiment to export PDF.

A duty time CSV file is also generated for every run.

The report PDF and CSV files are saved to the same folder as the .fast5 and .fastq read files e.g. :\data\experiment\sample_ID\ for MinION Mk1B.

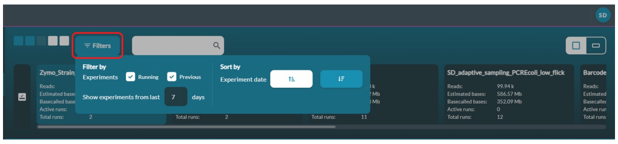




Select runs to export PDFs

X3 - DualTeth_OT - 21/04/2021, 14:03:31
X1 - DualTeth_FLT - 21/04/2021, 14:03:30
X2 - DualTeth_FLT - 21/04/2021, 14:03:30
X4 - DualTeth_OT - 21/04/2021, 14:03:30

To access previous sequencing runs and view them on the UI, click 'Filters' and select how many days previous to show past experiments.



Filter by
Experiments ☑ Running ☑ Previous
Show experiments from last 7 days
Sort by
Experiment date

SD_adaptive_sampling_PCREcoli_low_flick
Reads: 99.94 k
Estimated bases: 586.57 Mb
Basecalled bases: 352.09 Mb
Active runs: 0
Total runs: 12

END OF STEP

MinKNOW fallback folder

If writing out reads to your specified location fails (e.g. if the disk becomes full, or the connection is disrupted), the experiment will continue, and the reads will be written into a fallback folder:

Windows

.fast5 files: C:\data\reads\fallback (with the standard run data name sample ID as a folder)
FASTQ files: C:\data\reads\fastq_fallback (with the standard run data name sample ID as a folder)

Mac OS X:

.fast5 files: /Library/MinKNOW/data/fallback
FASTQ files: /Library/MinKNOW/data/fastq_fallback

Linux:

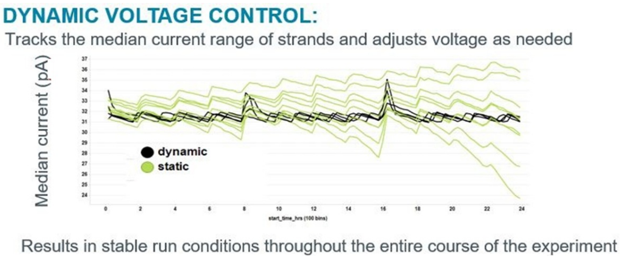
.fast5 files: /var/lib/MinKNOW/data/fallback
FASTQ files: /var/lib/MinKNOW/data/fastq_fallback

Adjusting the starting potential for multiple runs in series

Voltage drift during a sequencing run

Once the MinKNOW script proceeds to sequencing, the system goes through a cycle of voltage changes. The current scripts start with an applied voltage of -180 mV, which is optimal for basecalling with R9.4.1 chemistry. However in the process of a run, the voltage drifts due to the depletion of the redox chemistry in the bulk solution.

In order to keep the voltage constant, MinKNOW enables "Dynamic Voltage Control", which constantly monitors the raw signal from the device, and adjusts the applied voltage when required. Dynamic Voltage Control (in black) maintains a considerably tighter grouping of current levels, which is more stable over the course of a run than that previously achieved by the Static Voltage Control used in earlier versions of MinKNOW:



DYNAMIC VOLTAGE CONTROL:

Tracks the median current range of strands and adjusts voltage as needed

Results in stable run conditions throughout the entire course of the experiment

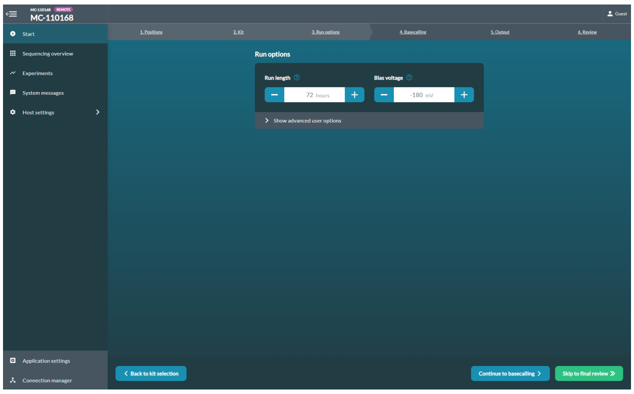
Adjusting the starting potential

If a flow cell is re-used (e.g. after running an experiment and washing the sample out with the Flow Cell Wash Kit), the common voltage will be lower than -180 mV due to the voltage drift. The exact voltage value will depend on the length of the experiment. To account for drift, the starting voltage has to be adjusted for the next run on the same flow cell using the following scheme:

Total previous runtime (hours)	Voltage to set (mV) for MinION Mk 1B, Flongle, or GridION	Voltage to set (mV) for PromethION
12	–190	–180
24	–210	–200
36	–230	–220
48 or more	–250	–240

To adjust the voltage, the user will need to edit the settings before starting a sequencing run.

Select **Start Sequencing** of the homepage and navigate to **Run Options** to alter the bias voltage by selecting and typing or using the **+** and **—** options to the appropriate voltage.



Run length: 72 hours

Bias voltage: -180 mV

Refuelling your flow cell

IMPORTANT

Refuelling: general advice

Refuelling is the replenishment of motor fuel in the sequencing experiment through the addition of Flush Buffer (FB) from the Flow Cell Priming Kit (EXP-FLP002). The translocation speed graph in MinKNOW can indicate whether it is necessary to top up fuel. Volumes and processes of refuelling are specific to the flow cell type. Please follow the instructions relevant to your flow cell type e.g. Flongle™, MinION™ or PromethION™.

When to refuel

If the DNA translocation speed drops below 300 bases per second, you may start to see a reduction in quality of data reflected in the Qscore. We therefore recommend topping up the flow cell with fuel, using the Flush Buffer (FB) from the Flow Cell Priming Kit. Please follow the instructions below if you wish to top up the fuel during an experiment.

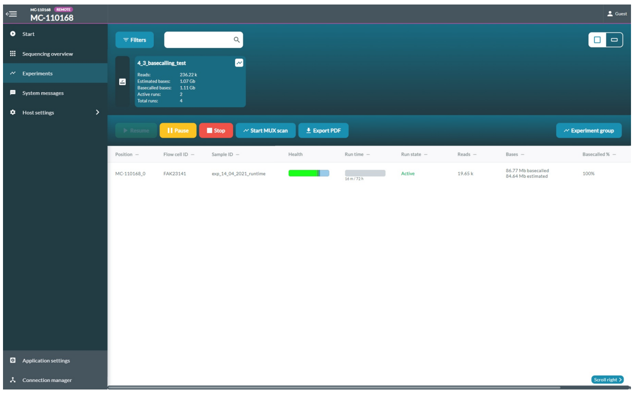
TIP

Please consult the 'Priming and loading the [SpotON/Flongle/PromethION] flow cell' step from the relevant library preparation protocol for advice on the adding solutions to the platform flow cell type that will be used.

Refuelling a Flongle™ flow cell

- Remove one tube of FB from the freezer and thaw by bringing to room temperature
- Pause the experiment on the GridION position/on the MinION Mk 1B Flow Cell that is being refuelled:

a. Navigate to **Experiments** and open the experiment running
b. Click **Pause**
c. A dialogue box will open. Choose the flow cell to pause and click **Pause** to confirm



4_3_basecalling_test

Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
MC-110168_0	FAK23141	exp_14_04_2021_runtime		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%

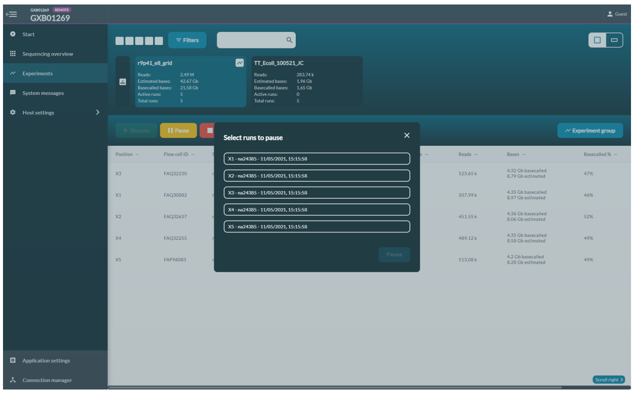
- Pull back the seal tab to reveal the sample port
- Pass 30 µl of FB through **sample port**
- Reseal the tab, ensuring the drain ports are covered
- Unpause the experiment on the relevant GridION position/on the MinION Mk 1B by clicking **Resume**
- *(optional)* Click **Start Mux Scan** to pick a new set of the best channels for the remainder of the sequencing experiment

Refuelling a MinION™/GridION™ flow cell

Instructions for refuelling a MinION/GridION flow cell:

- Remove one tube of Flush Buffer (FB) from the freezer and thaw by bringing to room temperature
- Pause the experiment on the GridION position/on the MinION Mk1B Flow Cell that is being refuelled:

a. Navigate to **Experiments** and select the experiment running
b. Click **Pause**
c. A dialogue box will open. Choose the flow cell to pause and click **Pause** to confirm



r9p41_e8_grid

Reads: 2.49 M
Estimated bases: 42.67 Gb
Basecalled bases: 21.58 Gb
Active runs: 5
Total runs: 5

TT_Ecoli_100521_JC

Reads: 283.74 k
Estimated bases: 1.96 Gb
Basecalled bases: 1.65 Gb
Active runs: 0
Total runs: 5

Select runs to pause

X1 - na24385 - 11/05/2021, 15:15:58
X2 - na24385 - 11/05/2021, 15:15:58
X3 - na24385 - 11/05/2021, 15:15:58
X4 - na24385 - 11/05/2021, 15:15:58
X5 - na24385 - 11/05/2021, 15:15:58

Position	Flow cell ID	Reads	Bases	Basecalled %
X3	FAQ32230	523.65 k	4.32 Gb basecalled 8.79 Gb estimated	47%
X1	FAQ30882	507.99 k	4.35 Gb basecalled 8.97 Gb estimated	46%
X2	FAQ32637	451.55 k	4.36 Gb basecalled 8.06 Gb estimated	52%
X4	FAQ32255	489.12 k	4.35 Gb basecalled 8.58 Gb estimated	49%
X5	FAP96083	513.08 k	4.2 Gb basecalled 8.28 Gb estimated	49%

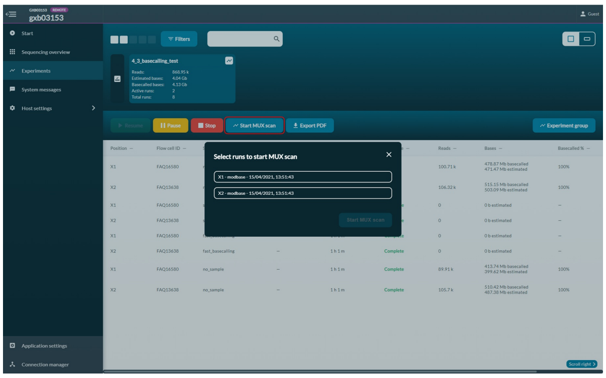
- After opening the priming port, check for a small bubble under the cover. Draw back a small volume to remove the bubble (a few µl):

a. Set a P1000 pipette to 200 µl
b. Insert the tip into the priming port
c. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip

Visually check that there is continuous buffer from the priming port across the sensor array.

Complete the flow cell refuelling:

- Load 250 µl of the FB into the flow cell via the **priming port** (i.e. **not** the **SpotON port**), avoiding the introduction of air bubbles
- Close the priming port and replace the MinION Mk1B/GridION lid
- Unpause the experiment on the relevant GridION position/on the MinION Mk1B by clicking **Resume**
- *(optional)* Click **Start Mux Scan** and choose your flow cell, to pick a new set of the best channels for the remainder of the sequencing experiment



Select runs to start MUX scan

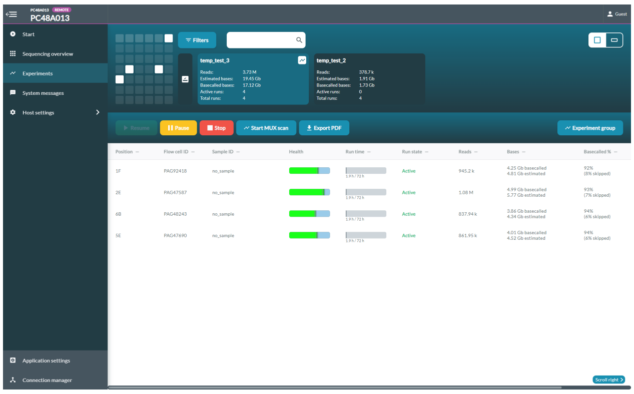
X1 - modbase - 15/04/2021, 13:51:43

X2 - modbase - 15/04/2021, 13:51:43

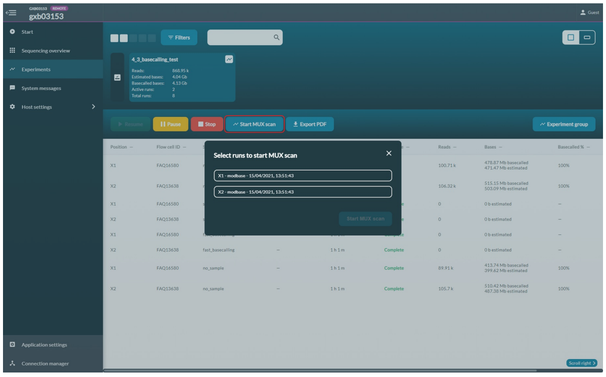
Refuelling a PromethION™ flow cell

- Remove one tube of FB from the freezer and thaw by bringing to room temperature
- Open the valve to reveal the inlet port
- Pause the experiment on the relevant PromethION Flow Cell position(s) that will be refuelled:

  a. Navigate to **Experiments** and open the experiment running
  b. Click **Pause**
  c. A dialogue box will open. Choose the flow cell to pause and click **Pause** to confirm



- Set a P1000 pipette to 200 µl
- Insert the tip into the **inlet port**
- Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip.
- Using a P1000 pipette, load 250 µl of the FB into the inlet port of the flow cell, avoiding the introduction of air bubbles
- Close the valve
- Incubate the flow cell with the added FB for 3 mins
- Unpause the experiment on the relevant PromethION Flow Cell position(s) after this process is complete by clicking **Resume**
- *(optional)* Click **Start Mux Scan** and choose your flow cell, to pick a new set of the best channels for the remainder of the sequencing experiment



Select runs to start MUX scan

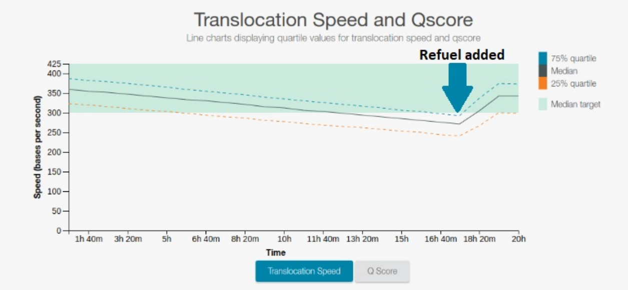
X1 - modbase - 15/04/2021, 13:51:43

X2 - modbase - 15/04/2021, 13:51:43

The outcome and benefits of refuelling

Translocation speed and Qscore over time

Below is a graph that shows what is expected for translocation speed after the addition of FB to the flow cell in a previous version of MinKNOW.



Speed of bases through nanopores before and after refuelling

As the speed drops below 300 bases per second, the Qscore will begin to decline for the reads processed through the nanopores at this speed. After refuelling at the 17.5 hour mark, the speed begins to increase and returns to an improved rate (~400 bases per second), which is similar to the speed at the start of the experiment. After the addition of fuel using FB, the quality of the data may increase and return to Qscores equivalent to those seen at the start of your run.

Refuelling multiple times in a run

You can refuel a sequencing run multiple times over an experiment. When you should refuel will depend on when the translocation speed drops below 300 bases per second on the speed graph in the MinKNOW GUI.

CAUTION

Warning - overloading the flow cell when refuelling

If you refuel your flow cell multiple times, the waste reservoir of the flow cell will gradually fill up with buffer.

The array and waste reservoir of the below platforms (Flongle, MinION Mk 1B/GridION, PromethION) will take the following fluid loads:

- a prime
- a library
- **one** refuel - for Flongle
- **three** refuels - for MinION/GridION
- **four** refuels - for PromethION

Checks and monitoring

During the sequencing experiment, you can check various flow cell health and performance parameters that are shown in the MinKNOW GUI. Each parameter is described in more detail in this section.

Flow cell health

During a sequencing experiment, the MinKNOW Sequencing Overview page shows a flow cell icon with coloured bars. The bars represent the combined health of all pores in a flow cell, and indicate how well the flow cell is performing. The colours are:

- Light green: sequencing
- Dark green: open pore
- Dark blue: pore recovering
- Light blue: pore inactive

This information is identical to the last bar of the duty time plot (described later).

Flongle/MinION/GridION flow cell health diagram

Note: The below image illustrates flow cell health on a GridION. A MinION device will only have one flow cell.

PromethION flow cell health diagram

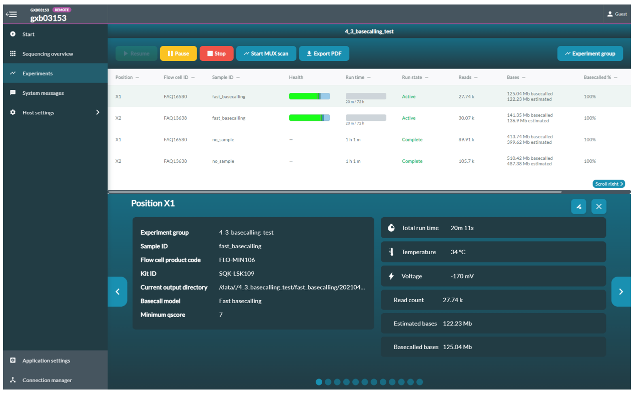
1A No flow cell detected	2A No flow cell detected	3A PAH00826 Sequencing	4A PAG95735 Sequencing	5A No flow cell detected	6A No flow cell detected
1B No flow cell detected	2B No flow cell detected	3B PAH01508 Sequencing	4B PAH03301 Sequencing	5B No flow cell detected	6B PAG48243 Sequencing
1C No flow cell detected	2C No flow cell detected	3C PAH04560 Sequencing	4C PAG94337 Sequencing	5C No flow cell detected	6C No flow cell detected
1D No flow cell detected	2D No flow cell detected	3D PAH01750 Sequencing	4D PAG95043 Sequencing	5D No flow cell detected	6D No flow cell detected
1E No flow cell detected	2E PAG47587 Sequencing	3E PAH05709 Sequencing	4E PAG94274 Sequencing	5E PAG47690 Sequencing	6E No flow cell detected
1F PAG92418 Sequencing	2F No flow cell detected	3F PAH04389 Sequencing	4F PAG95164 Sequencing	5F No flow cell detected	6F No flow cell detected
1G No flow cell detected	2G No flow cell detected	3G PAH05539 Sequencing	4G PAG95470 Sequencing	5G No flow cell detected	6G No flow cell detected
1H No flow cell detected	2H No flow cell detected	3H PAH04378 Sequencing	4H PAG95453 Sequencing	5H No flow cell detected	6H No flow cell detected

Experiment summary information

The Experiments page displays summary information for all sequencing flow cells and device checks carried out on the device.

From this page, the user is able to control specific runs and identify real-time information including flow cell health and reads, giving users real-time feedback for sequencing flow cells.

- **Run statistics:** The total number of reads and bases produced across the experiment
- **Basecall statistics:** There are two values for basecalled reads:
  1. Basecalled reads as a percentage of the total reads produced across the experiment. This gives an indication as to the size of the queue for reads to be basecalled
  2. Total number of reads basecalled across the experiment
- **Run time:** The duration of the experiment
- **Temperature:** The heatsink temperature of the selected position, which should reach 34°C (or 36°C on Flongle) for sequencing
- **Voltage:** The applied potential of the position at that point in time



4_3_basecalling_test

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		20 m / 72 h	Active	27.74 k	125.04 Mb basecalled 122.23 Mb estimated	100%
X2	FAQ13638	fast_basecalling		20 m / 72 h	Active	30.07 k	141.35 Mb basecalled 136.9 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

Position X1

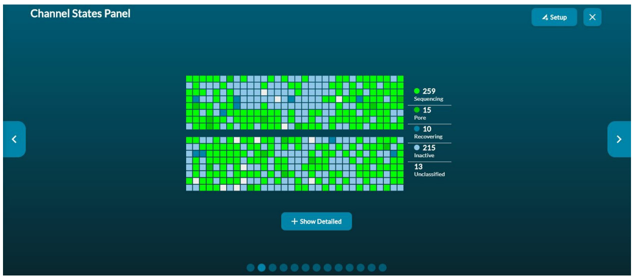
Experiment group 4_3_basecalling_test
Sample ID fast_basecalling
Flow cell product code FLO-MIN106
Kit ID SQK-LSK109
Current output directory /data/./4_3_basecalling_test/fast_basecalling/202104...
Basecall model Fast basecalling
Minimum qscore 7

Total run time 20m 11s
Temperature 34 °C
Voltage -170 mV
Read count 27.74 k
Estimated bases 122.23 Mb
Basecalled bases 125.04 Mb

> **TIP**
>
> **As the MinKNOW script progresses, you can check the following:**
>
> - Number of active pores
> - Heatsink temperature
> - Development of the read histogram
> - Pore occupancy
> - Local basecalling report

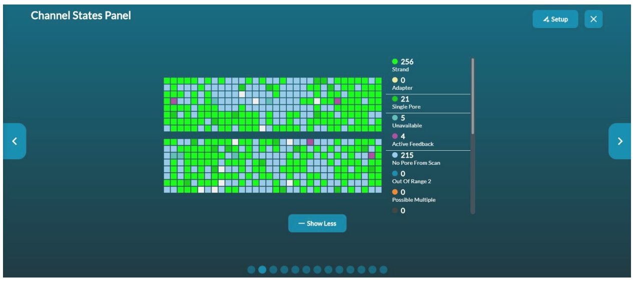
Pore occupancy

- A good library will be indicated by a higher proportion of light green channels in **Sequencing** than are in **Pore**. The combination of Sequencing and Pore indicates the number of active pores at any point in time. A low proportion of Sequencing channels will reduce the throughput of the run.
- **Recovering** indicates channels that may become available for sequencing again. A high proportion of this may indicate additional clean up steps are required during your library preparation.
- **Inactive** indicates channels that are no longer available for sequencing. A high proportion of these as soon as the run begins may indicate an osmotic imbalance.
- **Unclassified** are channels that have not yet been assigned one of the above classifications.



Clicking on the **Show Detailed** button reveals a more detailed array of channel states:

- **Strand:** the channel has strand
- **Adapter:** the pore is sequencing the unligated sequencing adapter only. Reads will initially be classified as adapter until the DNA/RNA strand starts translocating through the pore and MinKNOW™ is able to reclassify the read
- **Single pore:** the channel appears to show a single pore. Available for sequencing
- **Unavailable:** the channel appears to show a pore that is currently unavailable for sequencing
- **Active feedback:** the channel is reversing the current flow to eject the analyte
- **No pore from scan:** the Mux scan has not detected a pore in the well
- **Out of range 2:** current level is between 10 and 9999 pA. Currently unavailable for sequencing
- **Possible multiple:** the channel appears to show more than one pore. Unavailable for sequencing
- **Saturated:** the channel has switched off due to current levels exceeding hardware limitations
- **Out of range 1:** current level is between -5 and -9999 pA. Currently unavailable for sequencing
- **Zero:** Current level is between -5 and 10 pA. Currently unavailable for sequencing

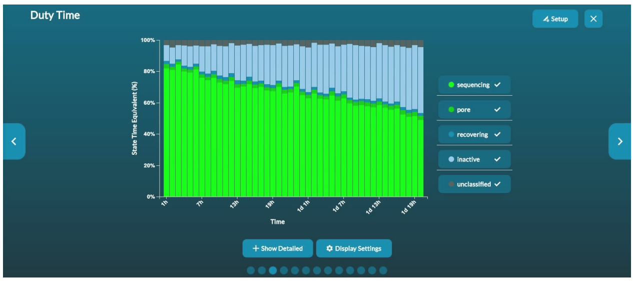


Duty time plots

The duty time plot summarises the channel states over time.

Each bar shows the sum of all channel activity in a particular amount of time. This time bucket defaults to 1 minute, and scales to 5 minutes automatically after reaching 48 buckets. However, bucket size can be adjusted in the "Bucket size" box in **Display Settings**.

The graph populates over time, and can be used as a way to assess the quality of your sequencing experiment, and make an early decision whether to continue with the experiment or to stop the run.



Read length histogram

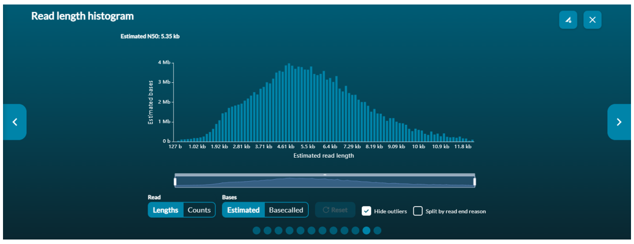
The cumulative histogram shows reads compared to bases. Use the options below to choose the axis legends:

- Y-axis: Estimated bases or basecalled bases
- X-axis: Read length or read counts

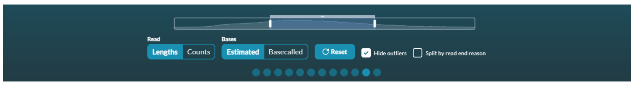
Read count - this shows the number of reads vs read length. This enables the user to understand how the read lengths vary in number and size.

Read length - this shows the total number of bases vs the read length.

The N50 value is presented (only for the whole set of passed reads) in the top left corner of the histogram.



Each histogram's X-axis (read length) can be zoomed in using the scaled bar under the histogram. Use **Reset** to refocuse the zoom bar and histogram for the entire 'passed read' dataset.

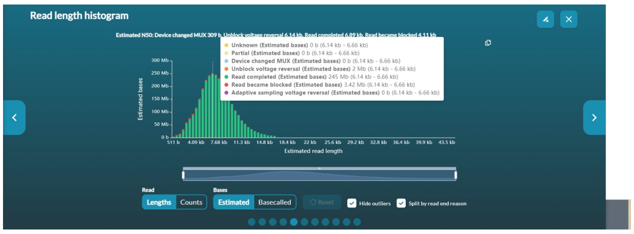


You can see the number of bases by hovering over the bar in question.

Reads which are outliers in terms of length can be removed from the graph by ticking the **Hide outliers** box below the histogram.

Select **Split by read end reason** to view split reads and hover over for further information. This is useful for adaptive sampling which is further explained in the Adaptive Sampling info sheet.

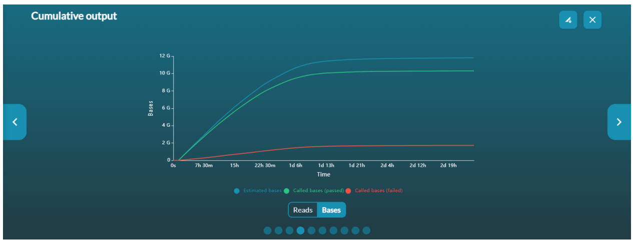
- **Device changed MUX:** The strand ended because there was a scheduled MUX change that interrupted the strand
- **Unblock voltage reversal:** The strand ended by a downtick in the signal
- **Read completed:** The strand ended naturally (there was an uptick in the signal, which is an indication of a pore)
- **Read became blocked:** The strand ended because it was deemed of low quality and purposefully rejected
- **Adaptive sampling voltage reversal:** The strand was rejected by adaptive sampling (typically happens after ~500 bp)



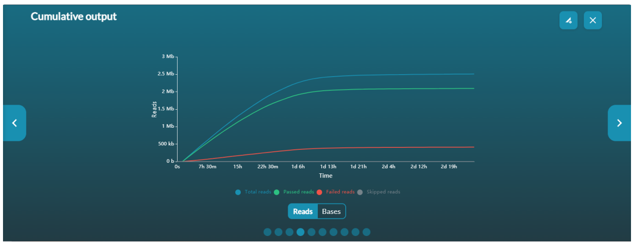
Cumulative output

The cumulative output graph shows:

- the number of bases that have been sequenced and basecalled



- the number of reads that have been sequenced and basecalled; and whether the reads have passed of failed the quality filters

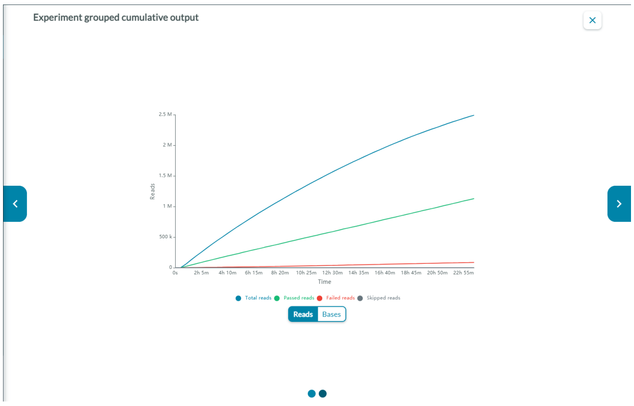


Cumulative output of multiple flow cells

If there are multiple flow cells running under the same experiment name, you will see the **Experiment view**. This gives outhput information on all assigned flow cells, plus a running cumulative total of *bases* or *reads* sequenced.

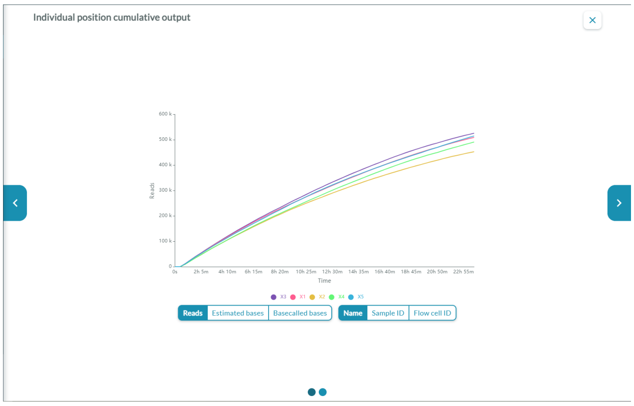
Total (cumulative) output vs time

The cumulative output graph shows the running total number of Gbases sequenced by the multi-flow cell platforms, like GridION™ and PromethION™.



Multi-flow cell output vs time

The output generated by each flow cell to make the total cumulative output can be represented by individual output plots (different coloured lines on the graph below).

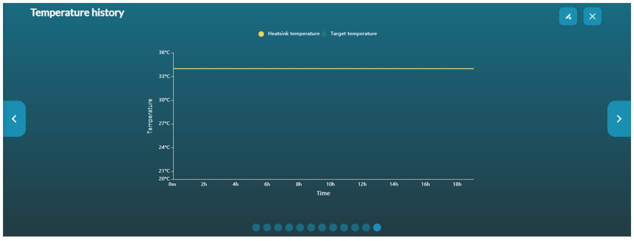


Both graphs can be switched between in the MinKNOW GUI. Reads, bases (estimated or basecalled) depending on the name, sample ID or flow cell ID can be selected to tailor the output graph, as required.

Temperature and Bias Voltage graph

Temperature vs time graph

The temperature graph gives a real-time representation of the temperature below the flow cell. If the temperature reading drifts out of the target zone, please consult Technical Services, otherwise the quality of your data may be compromised.

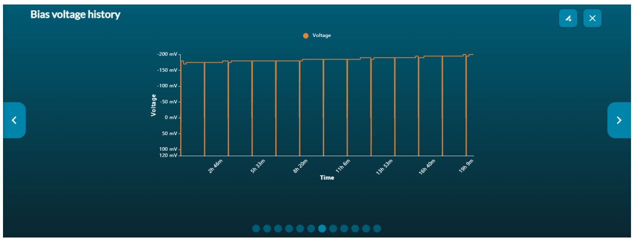


Bias voltage vs time graph

The bias voltage graph provides the running voltage in real-time. MinKNOW will automatically adjust the applied voltage and will naturally drift to lower voltages as the electrochemistry in the flow cell is depleted. This graph is useful for running a flow cell multiple times.

If you set the voltage for a subsequent run as the final running voltage of the previous run, then MinKNOW will find it easier to identify the appropriate running voltage.

You will notice drops in the voltage at regular intervals and these will correspond to the MUX scans that are defaulted to occur every one and a half hours. Here, each MUX - the options available to each channel - will be scanned to look for its availability for sequencing. The common voltage is reversed before and after each MUX assessment for clearer results.

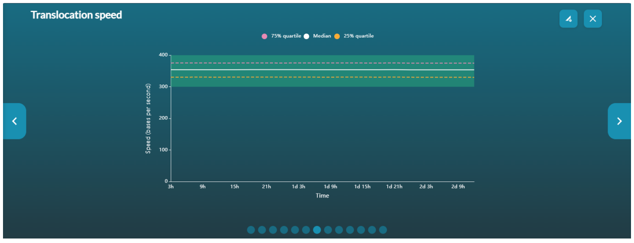


Translocation speed and Qscore graphs

Note: These graphs are only present if Basecalling is turned on.

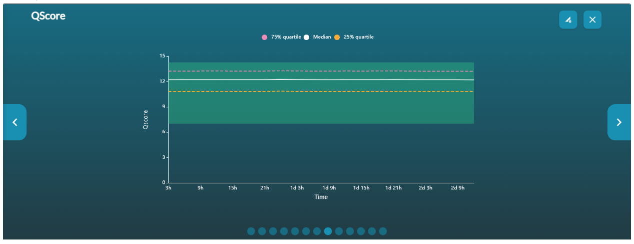
Translocation speed vs time

The translocation graph gives a real-time representation of the speed at which DNA/RNA strands pass through the pore. If the translocation speed drops below this window, data quality and output may be compromised as strands take longer to move through the pore.



Qscore vs time

The Qscore graph gives a live representation of the median strand Qscore over time.



Barcode read counts

The Barcode Read Counts graph shows the breakdown of barcoded reads, if barcoding was used for the experiment. The default view only shows reads that have passed the quality score filters. However, selecting the **Display failed** box will show all reads.

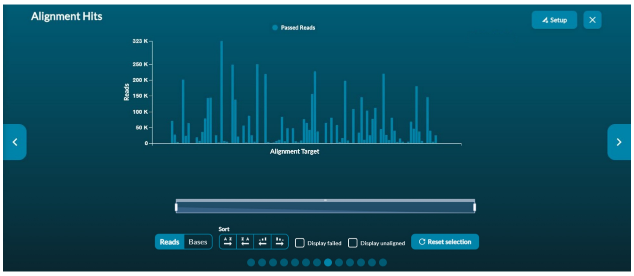
The X-axis of the histogram also has a zoom function using the scaled bar underneath. Use the **Reset** to refocus the zoom bar and graph.

Note that there is a small amount of cross-talk between barcodes. Some barcoded reads may appear on the graph even if the barcode in question was not used for the experiment.

Note: This graph is only present if barcoded is turned on.

Alignment hits

The alignment hits graph will populate when alignment and basecalling is set up to run during sequencing. This bar graph shows the number of reads and bases that align to each of the entries in the user reference .fasta file or minimap index file. An entry in the reference file will only appear on the graph once a single read has aligned to it.

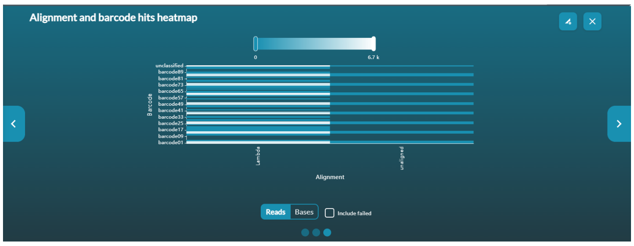


The X-axis of the histogram also has a zoom function using the scaled bar underneath. Use the **Reset** to refocus the zoom bar and graph.

Note: If a .bed file is used, the alignment hits graph will still display hits from the reference .fasta file or precompiled minimap index file. Currently, the .bed file is not used to populate the alignment graphs. However, the .bed file alignment hits will be highlighted in the sequencing summary .txt file generated in the data folder.

Alignment and barcode heatmap

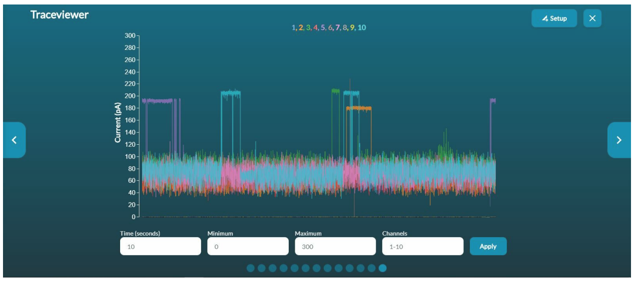
The alignment and barcode heatmap is only available when alignment and demultiplexing is performed with basecalling during sequencing. The heatmap graph shows the alignment hits split per barcode. The colour gradient shows bases and reads which are the more popular barcode and alignment hit combinations.
Use the options below to view reads or bases and the slide bar above to focus on specific regions.



Note: These graphs are only present if barcoding and an alignment is turned on.

Traceviewer

The Traceviewer displays the current levels from individual channels. By default, it is set to show 10 channels. This number can be changed through the selection boxes beneath the viewer. Additional parameters that can be altered:
- **Time:** The length of time plotted on one screen
- **Maximum:** The highest current level to be shown on the y axis



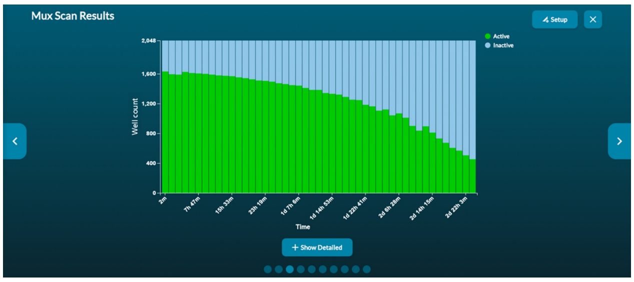
Time (seconds): 10
Minimum: 0
Maximum: 300
Channels: 1-10

Please note that viewing a high number of channels in the traceviewer may impact the speed at which the GUI is able to function.

Mux scan

As the sequencing protocol starts, a MUX scan begins before the sequencing. This multiplex scan allows MinKNOW to prioritise the order in which the nanopores are used, maximising the data output in the initial stages of the run. There are four groups of active pores, and group 1 are used in the first eight hours. So for the Lambda Control Experiment, only group 1 pores are used but for longer sequencing runs the other groups are used as required.

If Active Channel Selection is enabled during the run, the software instantly switches to a new channel in the group if a channel is in the “Saturated” or “Multiple” state, or after ~5 minutes if a channel is “Recovering”.



END OF STEP

End of sequencing protocol script.

The length of the sequencing scripts are indicated during script selection, e.g. 72 Hr. However, if live basecalling is selected, a minimum-spec laptop may not keep up with the speed of data acquisition, and not all reads will be basecalled by the end of the experiment. The reads that had not finished being basecalled during the experiment will continue to be basecalled in Catch-Up mode.

The reads from the experiment will be found in the location set during experiment set-up in MinKNOW. The read file structure is described here.
If the Desktop Agent is running simultaneously to MinKNOW, the full report will also be available. If it is being run at different times, the reads will be present in the data/reads folder ready to be processed.

Troubleshooting your run from the Duty Time plots

Duty Time plot introduction

The Duty Time feature in the MinKNOW software can be used to judge the quality of your experiment. The duty time plot shows the distribution of channel states over time, grouped by time chunks, or 'buckets'. The basic view shows the five main channel states: Sequencing, Pore, Recovering, Inactive, and Unclassified. Clicking the "More" button shows a more detailed breakdown of channel states.

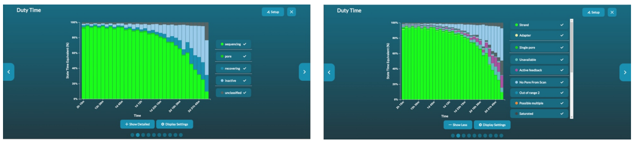
It is recommended to observe the duty time plot populating over the first 30 min-1 hr of the sequencing run. By this time, the channel state distribution will give an indication whether the DNA/RNA library is of a good quality, and whether the flow cell is performing well.

If Active Channel Selection is enabled during the run, the software instantly switches to a new channel in the group if a channel is in the “Saturated” or “Multiple” state, or after ~5 minutes if a channel is “Recovering”. This feature maximises the number of channels sequencing at the start of the experiment, however this may also result in an artificially high number of "Sequencing" or "Pore" channels in the duty time plot. For this reason, we recommend referring to the Mux Scan Results plot, which shows the true distribution of channel states at the point of the most recent mux scan.

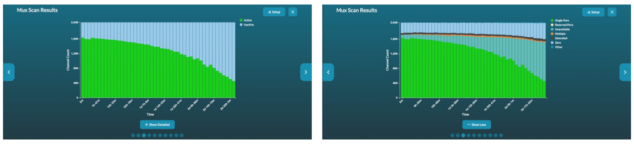
Good library

A good quality library will result in most of the pores being in the "Sequencing" state, and very few in "Pore", "Recovering" or "Inactive". A library that looks like this is likely to give a good sequencing throughput.

Duty time plot - basic and expanded channel views



Mux scan: basic and expanded channel states

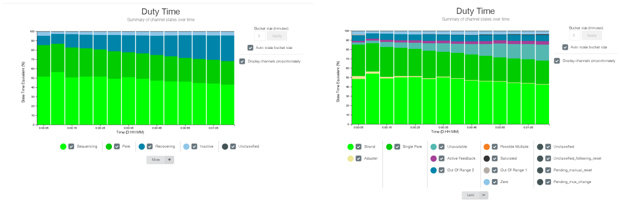


Channel blocking

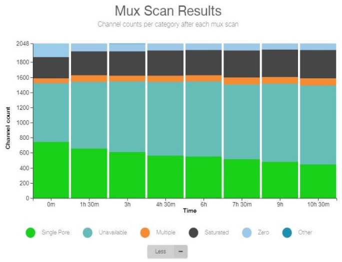
Under certain conditions (usually the presence of contaminants in the library), pores may become blocked and therefore unable to sequence. This manifests itself as a build-up of "Recovering" pores over time.

Note: Images are in the previous version of MinKNOW.

Duty time: basic and expanded channel states:



Mux scan results:



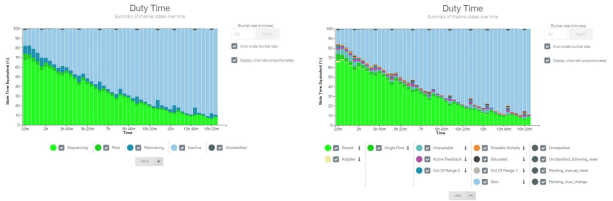
Recommendation:
- If, despite the channel blocking, the library is still producing a sufficient number reads to answer your biological question, you can carry on with the sequencing experiment.
- Otherwise, stop the sequencing run in MinKNOW. Then wash out the library from the flow cell using the instructions for the Flow Cell Wash Kit, which is included in your Starter Pack. Then prepare another library and load it on the flow cell.

Osmotic imbalance

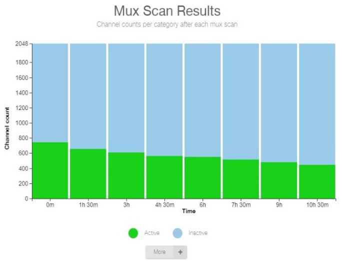
If the duty time plot shows a high number of 'Inactive' channels building up over time, this could indicate that the channels or membranes have been damaged by e.g. air bubbles, osmotic imbalance, or the presence of detergents or surfactants in the library.

Note: Images are in the previous version of MinKNOW.

Duty time: basic and expanded channel states



Mux scan:

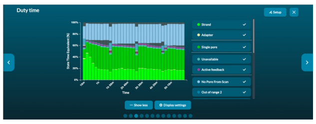


Recommendation:
- Check the channel panel: if the Inactive channels are all grouped in one part of the flow cell, this could indicate an air bubble that has been introduced during flow cell flushing or library loading. If the remaining channels are still sequencing, it is possible to carry on with the run. Do not try to move the air bubble, as this can damage even more channels.

If the Inactive channels are distributed throughout the flow cell:
- Check that the heat tape on the underside of the flow cell is intact.
- Make sure that the input DNA is in either TE buffer or nuclease-free water, and that the buffer contains no detergents or surfactants.
- Make a new batch of flow cell priming buffer (a mixture of Flush Buffer and Flush Tether). Flush the flow cell with the mixture, and load the library again.
- If during the flow cell check, the number of available pores was below 800, please contact Customer Support to replace your flow cell.

Low pore occupancy

If there was insufficient starting material, or some sample has been lost during library prep, or the sequencing adapters did not ligate well to the strand ends, the duty time plot will show a high ratio of "Pore" to "Sequencing" states, meaning that only a limited number of pores are sequencing at any one time.



Recommendation:

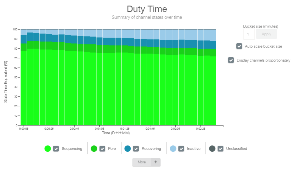
- Check the amount of DNA/RNA in your prepared library using e.g. the Qubit fluorometer. Then add another 200-300 ng of DNA/RNA to the existing run.
- If your library is at a low concentration, prepare the library again using a higher amount of starting material.

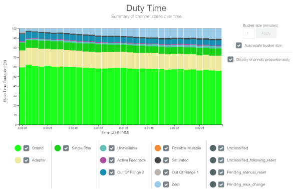
RNA duty time

For RNA in particular, the expanded channels view may show a large proportion of pores sequencing Adapter. This happens because RNA strands are usually shorter than DNA, and the adapter takes up a larger proportion of the strand. Additionally, the RNA sequencing chemistry is optimised for sequencing RNA, whereas the adapter is DNA, and is processed slower. As long as the 'basic' duty time plot view shows the majority of pores in "Sequencing", a high proportion of Adapter should not be a problem.

Note: Images are in the previous version of MinKNOW.

Duty time: basic and expanded channel states

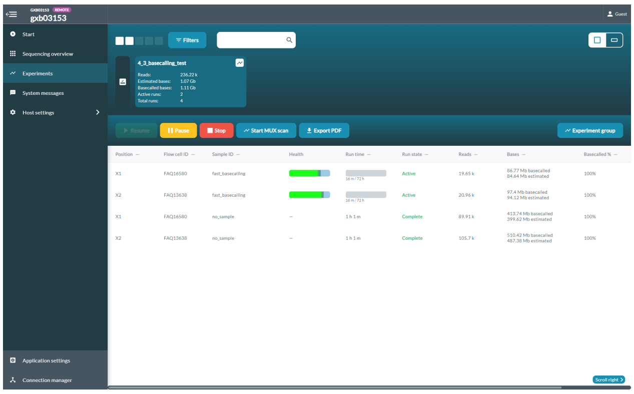




Completion of a sequencing protocol script

Catch-up notifications

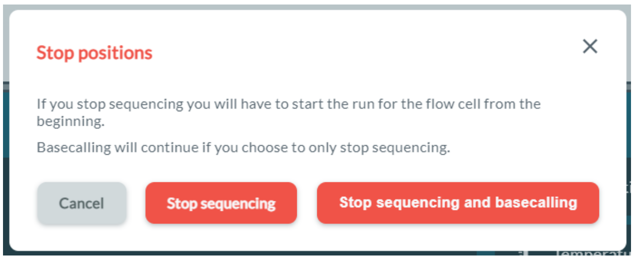
Catch-up basecalling is carried out either after the sequencing run completes (e.g. after 72 hours), or if the run is manually terminated. To manually terminate a run, select sequencing flow cells and click **Stop**.



4_3_basecalling_test
Reads: 236.22 k
Estimated bases: 1.07 Gb
Basecalled bases: 1.11 Gb
Active runs: 2
Total runs: 4

Position	Flow cell ID	Sample ID	Health	Run time	Run state	Reads	Bases	Basecalled %
X1	FAQ16580	fast_basecalling		16 m / 72 h	Active	19.65 k	86.77 Mb basecalled 84.64 Mb estimated	100%
X2	FAQ13638	fast_basecalling		16 m / 72 h	Active	20.96 k	97.4 Mb basecalled 94.12 Mb estimated	100%
X1	FAQ16580	no_sample	–	1 h 1 m	Complete	89.91 k	413.74 Mb basecalled 399.62 Mb estimated	100%
X2	FAQ13638	no_sample	–	1 h 1 m	Complete	105.7 k	510.42 Mb basecalled 487.38 Mb estimated	100%

A pop-up window will appear:



Stop positions

If you stop sequencing you will have to start the run for the flow cell from the beginning.

Basecalling will continue if you choose to only stop sequencing.

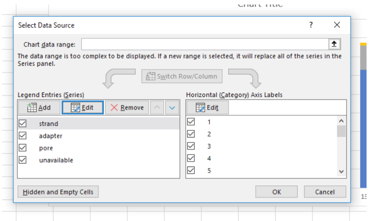
A progress bar is displayed during catch-up basecalling on the Sequencing Overview and Experiments pages.

> **IMPORTANT**
>
> **Please note that in the current version of MinKNOW, stopping your experiment and stopping basecalling through the GUI when ONLY FASTQ output is selected leads to queued reads being discarded. We are working on a fix.**

CSV files output by MinKNOW

The data used by MinKNOW to generate the Duty Time and Cumulative Output plots is output as CSV files. These files are located in the run-specific folder in the reports directory (e.g. \data\reports on Windows).

Using the data in the CSV files, users can recreate Duty Time and Cumulative Output graphs themselves in Excel by adding the various pore states to the Legend Entries:



The data range is too complex to be displayed. If a new range is selected, it will replace all of the series in the Series panel.

Acquisition termination file for LIMS

Customers using LIMS will receive a summary file once data acquisition and file writing has completed. The file is named *final_summary.txt*, and is written out to the output directory.

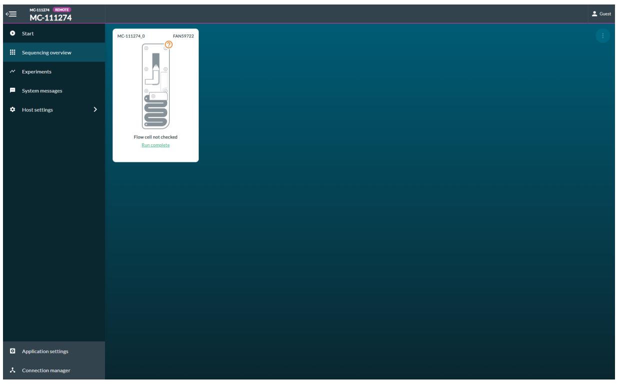
An example of the file contents is shown below:

```
instrument=PCT0009
position=1-A5-D5
flow_cell_id=PAD15520
sample_id=Chinese_NA24631
protocol_group_id=JB_47x_P48005_270319
protocol=4a37d56a62b6425a2af4d01d4970c7b5cc7b7c80
protocol_run_id=2a4ccaa1-8aaf-4872-a1f3-773a686d64de
acquisition_run_id=7eeaea330d1fe2ec43583072bbe21288254bef1b
started=2019-03-27T17:23:55.542284+00:00
acquisition_stopped=2019-03-28T09:58:37.505691+00:00
processing_stopped=2019-03-28T09:58:37.505788+00:00
basecalling_enabled=1
sequencing_summary_file=sequencing_summary/PCT0009_20190327_172337_PAD15520_promethion_sequencing_run_Chinese_NA24631_sequencing_summary.txt
fast5_files_in_final_dest=1606
fast5_files_in_fallback=0
fastq_files_in_final_dest=1197
fastq_files_in_fallback=0
```

Closing MinKNOW and shutting down a MinION Mk1B

~1 minutes

1 **Ensure that the flow cell is not running an experiment.**



2 **Close MinKNOW by closing down the GUI.**

3 **Disconnect the MinION Mk1B.**

Shutting down

Shutting down your GridION

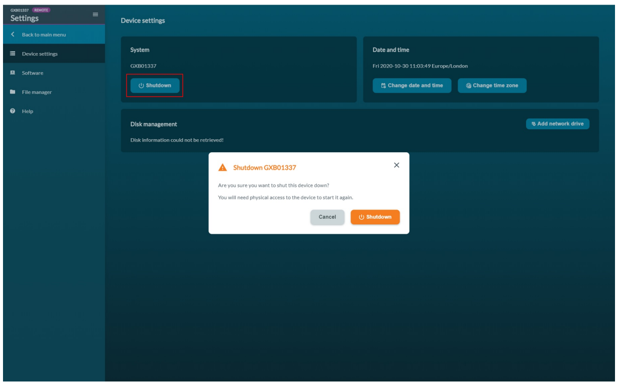
The GridION's computer requires a stepwise, processed shut down, otherwise you may face problems e.g. when recognising flow cells.

Follow the instructions in this section to ensure you do not face errors with your GridION from incorrectly shutting down your device.

1 **Ensure none of the flow cells are running an experiment.**

An indication no experiments are running is the lack of an experiment status bar beneath individual flow cells.

2 **Navigate to Device Settings and click 'Shutdown'.**



Fri 2020-10-30 11:03:49 Europe/London

Disk information could not be retrieved!

Are you sure you want to shut this device down?

You will need physical access to the device to start it again.

3 **Turn off the device at the mains supply, if you are using mains power.**

If remoting into a device using a computer not directly connected to a device, close MinKNOW by closing down the GUI.

Closing MinKNOW and shutting down on PromethION

~2 minutes

Shutting down your PromethION

The PromethION's computer requires a stepwise, processed shutdown, otherwise you may face problems e.g. when recognising flow cells.

Follow the instruction in this section to ensure you do not face errors with your PromethION from incorrectly shutting down your device.

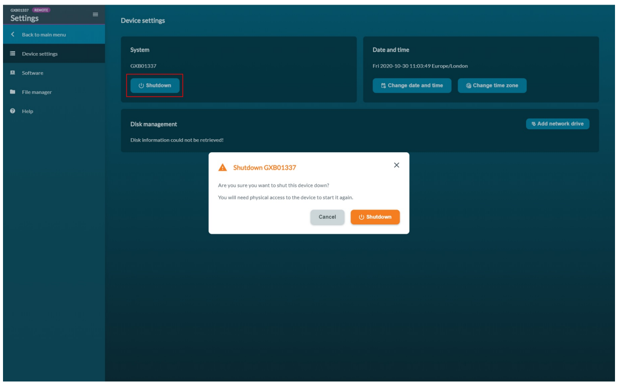
Post-run analysis

Version: MKE_1013_v1_revBZ_11Apr2016

1 **Ensure none of the flow cells are running an experiment.**

An indication no experiments are running is the lack of an experiment status bar beneath individual flow cells.

2 **Navigate to Device Settings and click 'Shutdown'.**



Disk information could not be retrieved!

Shutdown GXB01337

Are you sure you want to shut this device down?

You will need physical access to the device to start it again.

IMPORTANT

Do NOT select 'Reboot'. This will lead to errors in recognising the flow cells when commencing an experiment.

3 **Once the screen goes blank, turn off the sequencing module and tower via the switch at the rear of the device.**

If remoting into a device using a computer not directly connected to a device, close MinKNOW by closing down the GUI.

Post-run basecalling

Basecalling overview

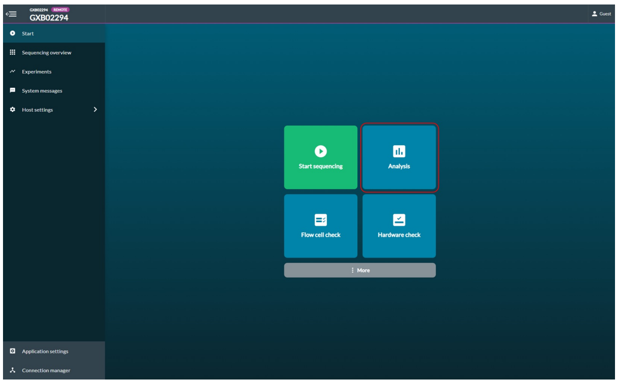
A user can basecall and demultiplex their data directly in MinKNOW after a sequencing experiment has finished to avoid having to use command-line tools, or to re-analyse old data using the latest basecalling models. Reads can also be aligned against a reference post-run.

Note: Both barcoding and alignment can be run on .fastq or .fast5 reads when coupled with basecalling.

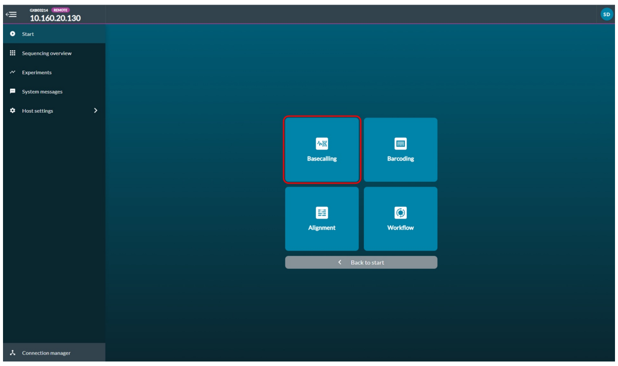
Post-run analysis

Version: MKE_1013_v1_revBZ_11Apr2016

1 **Select 'Analysis' on the start page.**



2 **Select 'Basecalling' to open the post-run basecalling set-up options.**

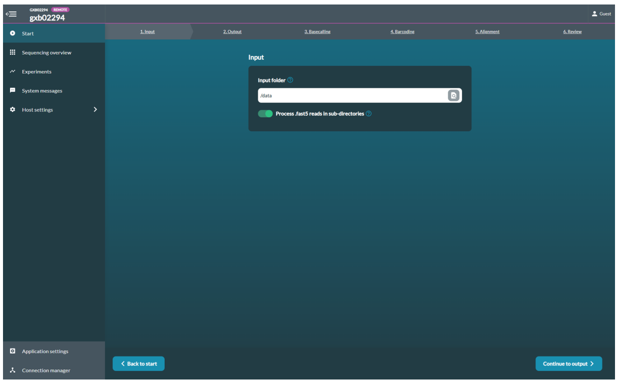


3 **Select input folder containing the .fast5 files to basecall from a previous run.**

Select whether you want to process .fast5 reads in sub-directories.

If your chosen read input folder contains sub-directories with .fast5 files, you can choose whether or not to basecall .fast5 files in these sub-directories by switching this option on.

Note: Data can only be saved to the `/data/` folder.



Input

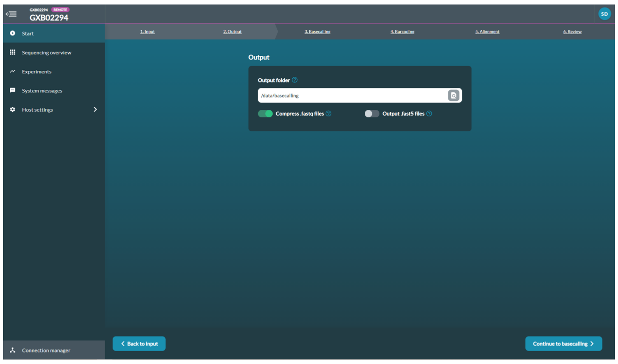
Input folder: /data

● Process .fast5 reads in sub-directories

4 **Select output folder and file type.**

By default, MinKNOW will create a `/basecalled` folder in `/data`. You can set a different folder which to save the basecalled reads.
Note: This must be a sub-folder of `/data` directory.

Select whether to output .fast5 files which will be copied into a folder within `/basecalled` called `/workspace`, if selected.



Output

Output folder: /data/basecalling

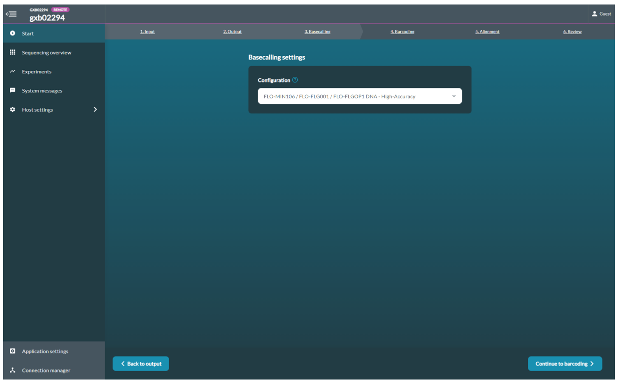
Compress .fastq files ●

Output .fast5 files ○

5 **Choose basecalling model from the drop-down menu.**

The model is used to process raw signal data for a basecall. The model name is composed of:

[flow cell product code] + [sample type] + [model type]
e.g. FLO-MIN106 / FLO-FLG001 - DNA - FAST



Basecalling settings

Configuration: FLO-MIN106 / FLO-FLG001 / FLO-FLGOP1 DNA - High-Accuracy

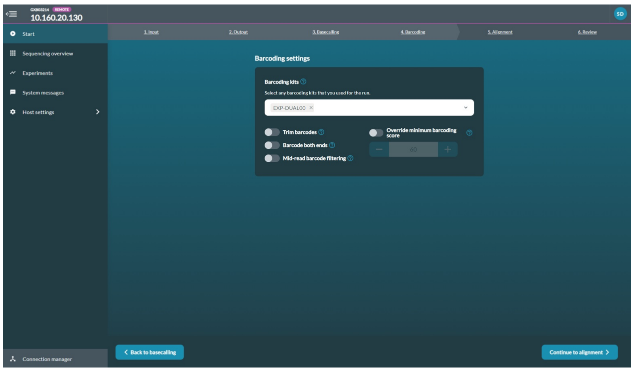
Given the recent improvements to basecalling accuracy, Q-score cut-off for reads put into the "pass" folder has been increased to:

Fast basecalling model >8
High accuracy (HAC) model >9
Super accurate (SUP) model >10

Optional Action
Select any barcoding kits used for the run from the drop down menu.

MinKNOW will write out basecalled reads into barcode-specific folders and enable demultiplexing during basecalling.

Users are able to choose specific settings, including trimming barcodes and minimum barcoding scores.



Barcoding settings

Barcoding kits
Select any barcoding kits that you used for the run.
EXP-DUAL00 ×

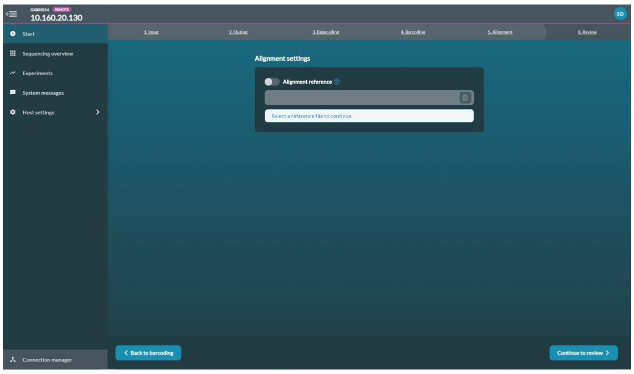
Trim barcodes ○
Barcode both ends ○
Mid-read barcode filtering ○
Override minimum barcoding score ○ 60

Optional Action
Select to use alignment and import a reference sequence in .fasta or a minimap index file.

We currently only recommend uploading an alignment reference locally on bacterial-sized genomes. Upload can take a few minutes and is compute-dependent.

A reference file must by uploaded as a .fasta or minimap index file which can contain multiple entries in the same file (e.g. multiple chromosomes).

However, a .bed file may also be uploaded alongside the reference .fasta or minimap index file. The .bed file option can be used when the user is interested in a particular region of the reference (e.g. specific gene in the chromosome).
Alignment generates .fastq output files when performed with basecalling.

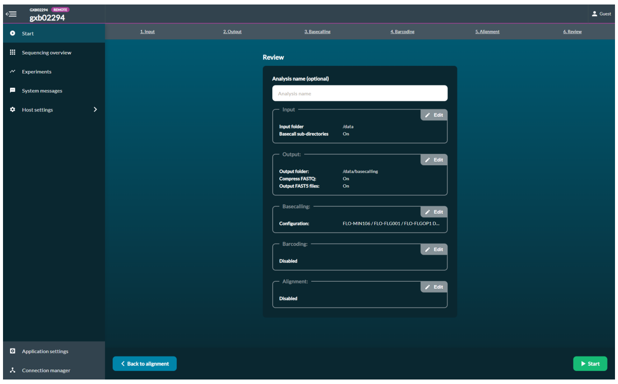


Alignment settings

Alignment reference

Select a reference file to continue.

6 **Select 'Start' to begin post-run basecalling.**



Review

Analysis name (optional)

Input
Input folder /data
Basecall sub-directories On

Output:
Output folder: /data/basecalling
Compress FASTQ: On
Output FAST5 files: On

Basecalling:
Configuration: FLO-MIN106 / FLO-FLG001 / FLO-FLGOP1 D...

Barcoding:
Disabled

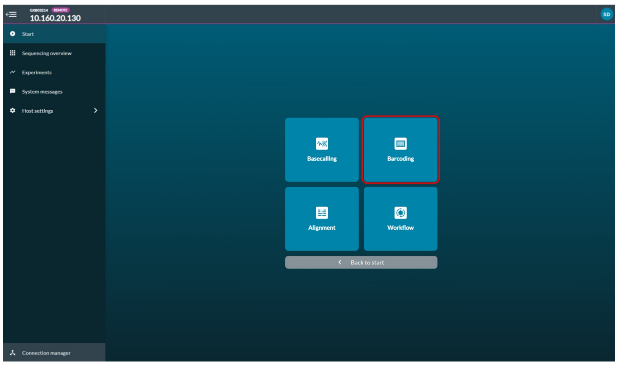
Alignment:
Disabled

Post-run barcoding

Barcoding overview

A user can barcode their data directly in MinKNOW after a sequencing experiment has finished, using .fastq data.

1 **Select 'Barcoding' in the analysis menu to open the post-run barcoding set-up options.**

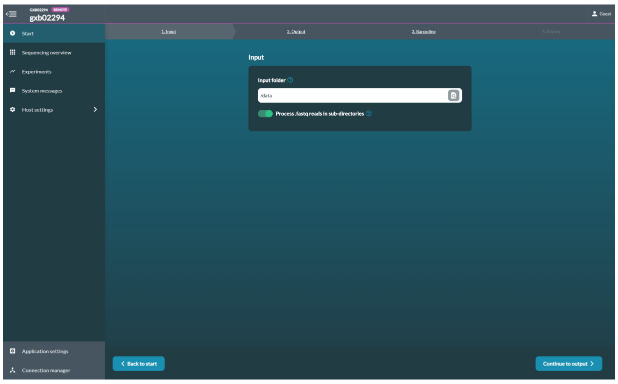


2 **Select the input folder containing the .fastq data to barcode from a previous run.**

Select whether you want to process .fastq reads in sub-directories.

If your chosen read input folder contains sub-directories with .fastq files, you can choose whether or not to barcode .fastq files in these sub-directories by swithcing this option on.

Note: Data can only be saved to the `/data` folder.



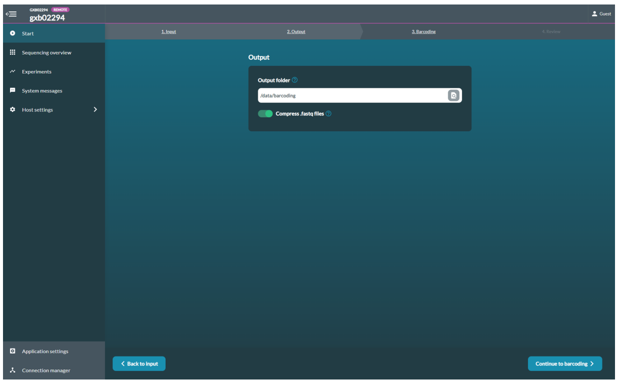
Input

Input folder: /data

☑ Process .fastq reads in sub-directories

3 **Choose output folder for the post-run barcoded data.**

By default, MinKNOW will create a `/barcoding` folder in `/data/`. You can set a different folder which to save the barcoded reads.
Note: This must be a sub-folder of `/data` directory.



Output

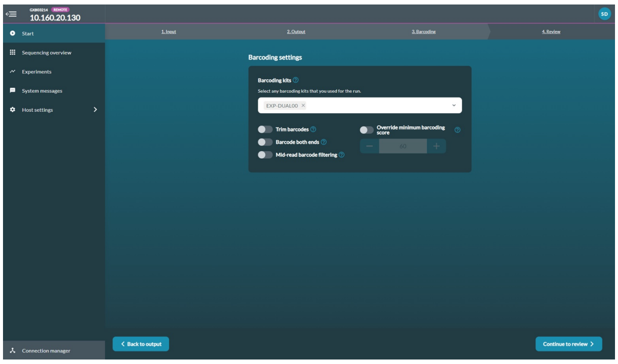
Output folder: /data/barcoding

☑ Compress .fastq files

4 **Select any barcoding kits used for the run from the drop down menu.**

MinKNOW will write out basecalled reads into barcode-specific folders and enable demultiplexing during basecalling.

Users are able to choose specific settings, including trimming barcodes and minimum barcoding scores.



Barcoding settings

Barcoding kits

Select any barcoding kits that you used for the run.

EXP-DUAL00

☐ Trim barcodes

☐ Barcode both ends

☐ Mid-read barcode filtering

☐ Override minimum barcoding score 60

5 **Select 'Start' to begin post-run barcoding.**

Post-run alignment

Alignment overview

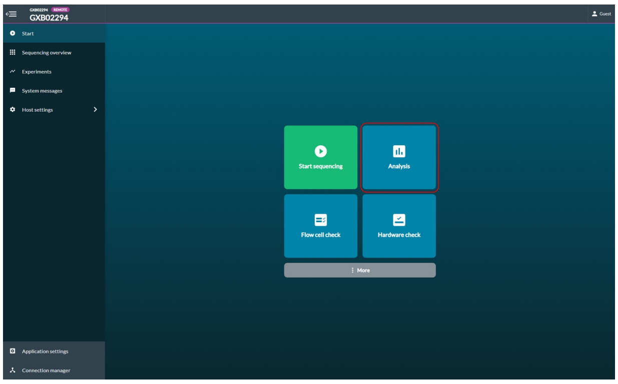
A user can align their data directly in MinKNOW after a sequencing experiment has finished, using a .fastq file from a previous run.

The reference file of bacterial-sized genomes must be uploaded locally as a .fasta file or minimap index file. Alignment hits from these files are used to populate the alignment graphs.

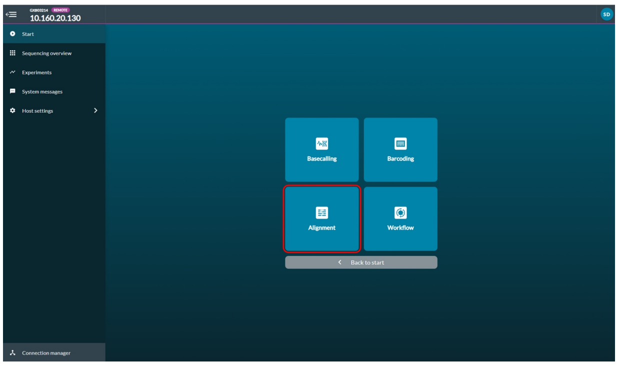
A .bed file may also be uploaded alongside the reference .fasta or minimap index file when the user is interested in a particular region of the reference (e.g. specific gene in the chromosome). The .bed file alignment hits will be highlighted in the sequencing .txt file generated in the data folder.

When alignment is run independently from basecalling, .bam files are generated.

1 **Select 'Analysis' on the start page.**



2 **Select 'Alignment' to open the post-run analysis set-up options.**

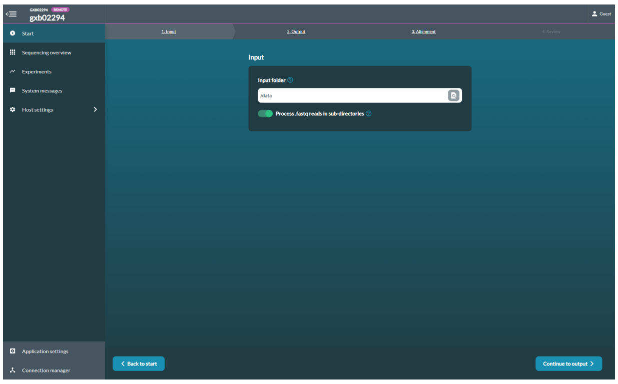


3 **Select the input file containing the .fastq data to carry out alignment from a previous run.**

Select whether you want to process .fastq reads in sub-directories.

If your chosen read input folder contains sub-directories with .fastq files, you can choose whether or not to align .fastq files in these sub-directories by switching this option on.

Note: Data can only be saved to the `/data` folder.



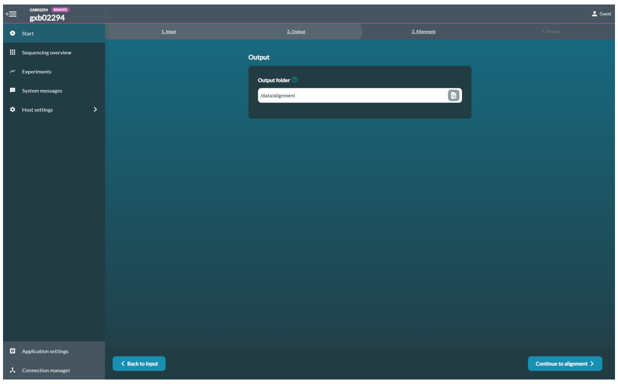
Input

Input folder: /data

● Process .fastq reads in sub-directories

4 **Choose the output folder for the post-run aligned data.**

By default, MinKNOW will create an `/alignment/` folder in `/data/`. You can set a different folder which to save the aligned reads.
Note: This must be a sub-folder of `/data` directory.



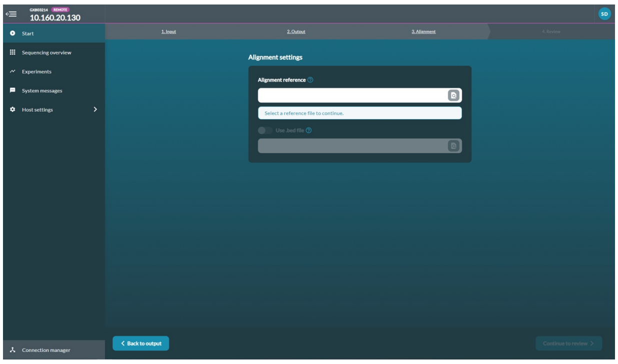
Output

Output folder: /data/alignment

5 **Select the alignment reference to carry out the post-run alignment.**

The alignment reference must either be a .fasta file or a pre-compiled minimap index file.

However, there is also the option to use a .bed file alignment reference as well.



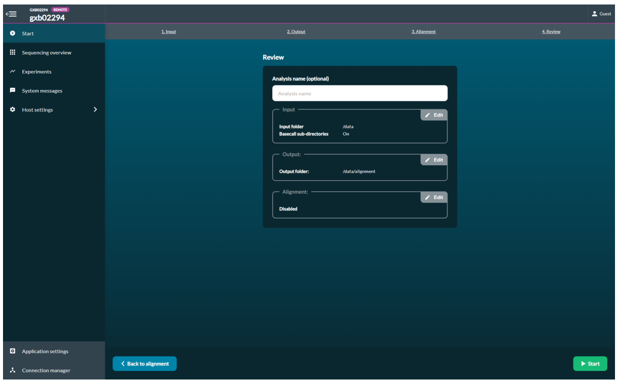
Alignment settings

Alignment reference

Select a reference file to continue.

Use .bed file

6 **Select 'Start' to begin post-run alignment.**



Review

Analysis name (optional)

Input
Input folder /data
Basecall sub-directories On

Output:
Output folder: /data/alignment

Alignment:
Disabled