Oxford Nanopore Technologies

GRIDION: PROTOCOL

Rapid Sequencing Kit V14 - gDNA (SQK-RAD114)

V RSE_9177_v114_revO_09Jan2025

The fastest and simplest protocol for genomic DNA involving:

- ~10 mins library prep
- Fragmentation
- No third-party ligase needed
- No PCR

This is an Early Access product For more information about our Early Access programmes, please see this article on product release phases.

For Research Use Only

FOR RESEARCH USE ONLY

Contents

1. Overview of the protocol

IMPORTANT

This is an Early Access product

For more information about our Early Access programmes, please see this article on product release phases.

Please ensure you always use the most recent version of the protocol.

Introduction to Rapid Sequencing Kit V14 (SQK-RAD114)

This protocol describes the step-by-step instructions to complete a rapid sequencing of genomic DNA using the Rapid Sequencing Kit V14 (SQK-RAD114). This protocol uses our most recent Kit 14 chemistry and is optimised for a fast library preparation and requires minimal laboratory equipment.

It is highly recommended that a Lambda control experiment is completed first to become familiar with the technology.

Steps in the sequencing workflow:

Prepare for your experiment

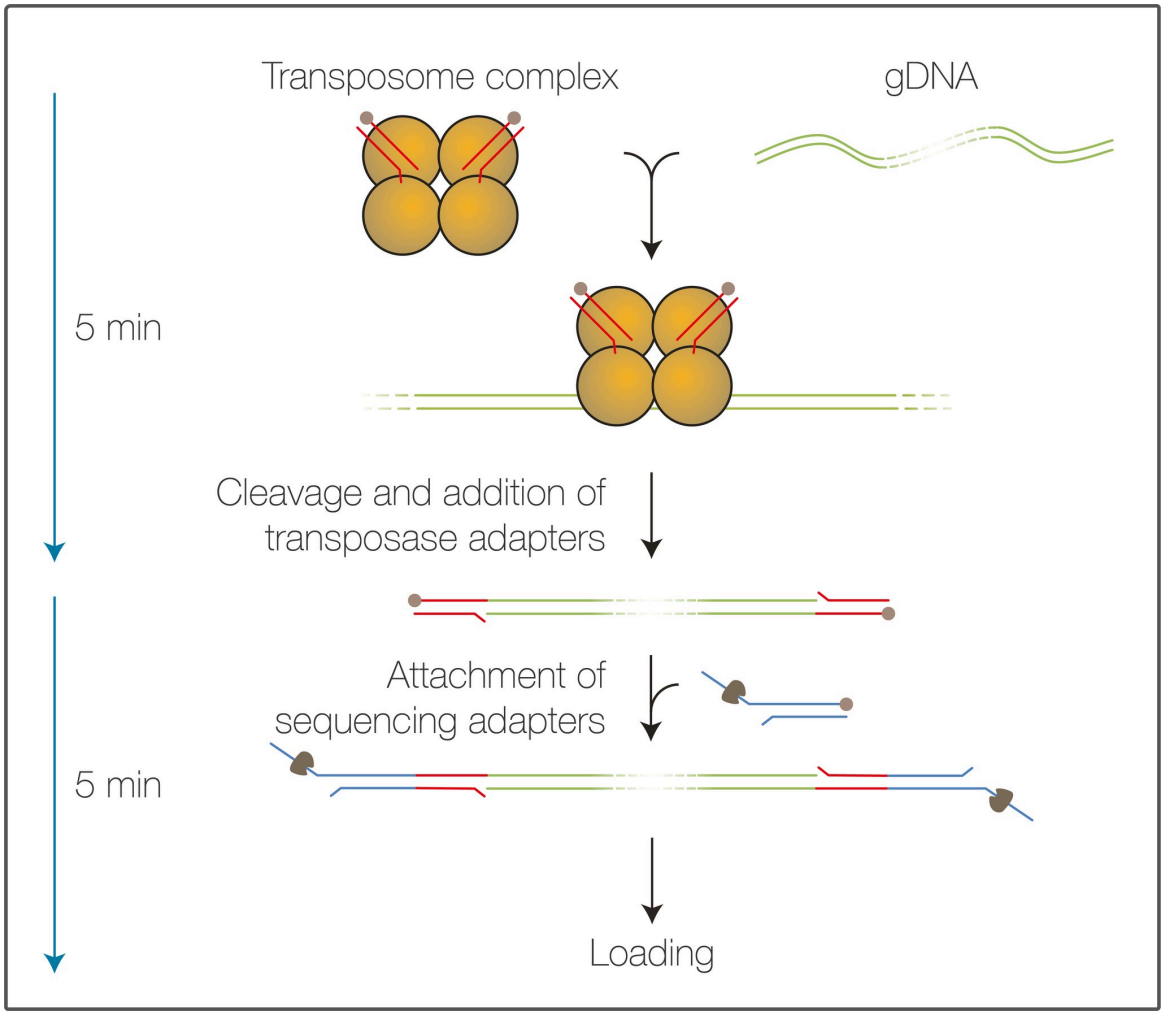
You will need to:

- Extract your DNA, and check its length, quantity and purity using the Input DNA/RNA QC protocol.
  The quality checks performed during the protocol are essential in ensuring experimental success.
- Ensure you have your sequencing kit, the correct equipment and third-party reagents
- If not already installed, download the software for acquiring and analysing your data
- Check your flow cell(s) to ensure it has enough pores for a good sequencing run

Library preparation

You will need to:

Library preparation step	Process	Time	Stop option
Tagmentation	Tagment your DNA using the Fragmentation Mix	5 minutes	-
Adapter attachment	Attach sequencing adapters to the DNA ends	5 minutes	We strongly recommend sequencing your library as soon as it is adapted.
Priming and loading the flow cell	Prime the flow cell and load the prepared library for sequencing	5 minutes	-



Sequencing and analysis

You will need to:

- Start a sequencing run using the MinKNOW software, which will collect raw data from the device and basecall reads.
- **Optional:** Start the EPI2ME software and select a workflow for further analysis, e.g. metagenomic analysis or drug resistance mapping.

IMPORTANT

Compatibility of this protocol

This protocol should only be used in combination with:

- Rapid Sequencing Kit V14 (SQK-RAD114)
- Control Expansion (EXP-CTL001)
- R10.4.1 MinION flow cells (FLO-MIN114)
- Flow Cell Wash Kit (EXP-WSH004)
- MinION Mk1B - MinION Mk1B IT requirements document
- MinION Mk1C - MinION Mk1C IT requirements document
- MinION Mk1D - MinION Mk1D IT requirements document
- GridION - GridION IT requirements document

2. Equipment and consumables

Materials	100-150 ng high molecular weight genomic DNA Rapid Sequencing Kit V14 (SQK-RAD114)
Consumables	MinION and GridION Flow Cell Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616) Nuclease-free water (e.g. ThermoFisher, AM9937) 1.5 ml Eppendorf DNA LoBind tubes 0.2 ml thin-walled PCR tubes
Equipment	MinION or GridION device MinION and GridION Flow Cell Light Shield Microfuge P1000 pipette and tips P100 pipette and tips P20 pipette and tips P10 pipette and tips P2 pipette and tips Timer Thermal cycler or heat blocks
Optional equipment	Qubit fluorometer (or equivalent for QC check)

For this protocol, you will need ~100 ng high molecular weight genomic DNA.

Lower inputs can be used but sequencing output will be reduced.

Input DNA

How to QC your input DNA

It is important that the input DNA meets the quantity and quality requirements. Using too little or too much DNA, or DNA of poor quality (e.g. highly fragmented or containing RNA or chemical contaminants) can affect your library preparation.

For instructions on how to perform quality control of your DNA sample, please read the Input DNA/RNA QC protocol.

Chemical contaminants

Depending on how the DNA is extracted from the raw sample, certain chemical contaminants may remain in the purified DNA, which can affect library preparation efficiency and sequencing quality. Read more about contaminants on the Contaminants page of the Community.

Third-party reagents

We have validated and recommend the use of all the third-party reagents used in this protocol. Alternatives have not been tested by Oxford Nanopore Technologies.

For all third-party reagents, we recommend following the manufacturer's instructions to prepare the reagents for use.

Check your flow cell

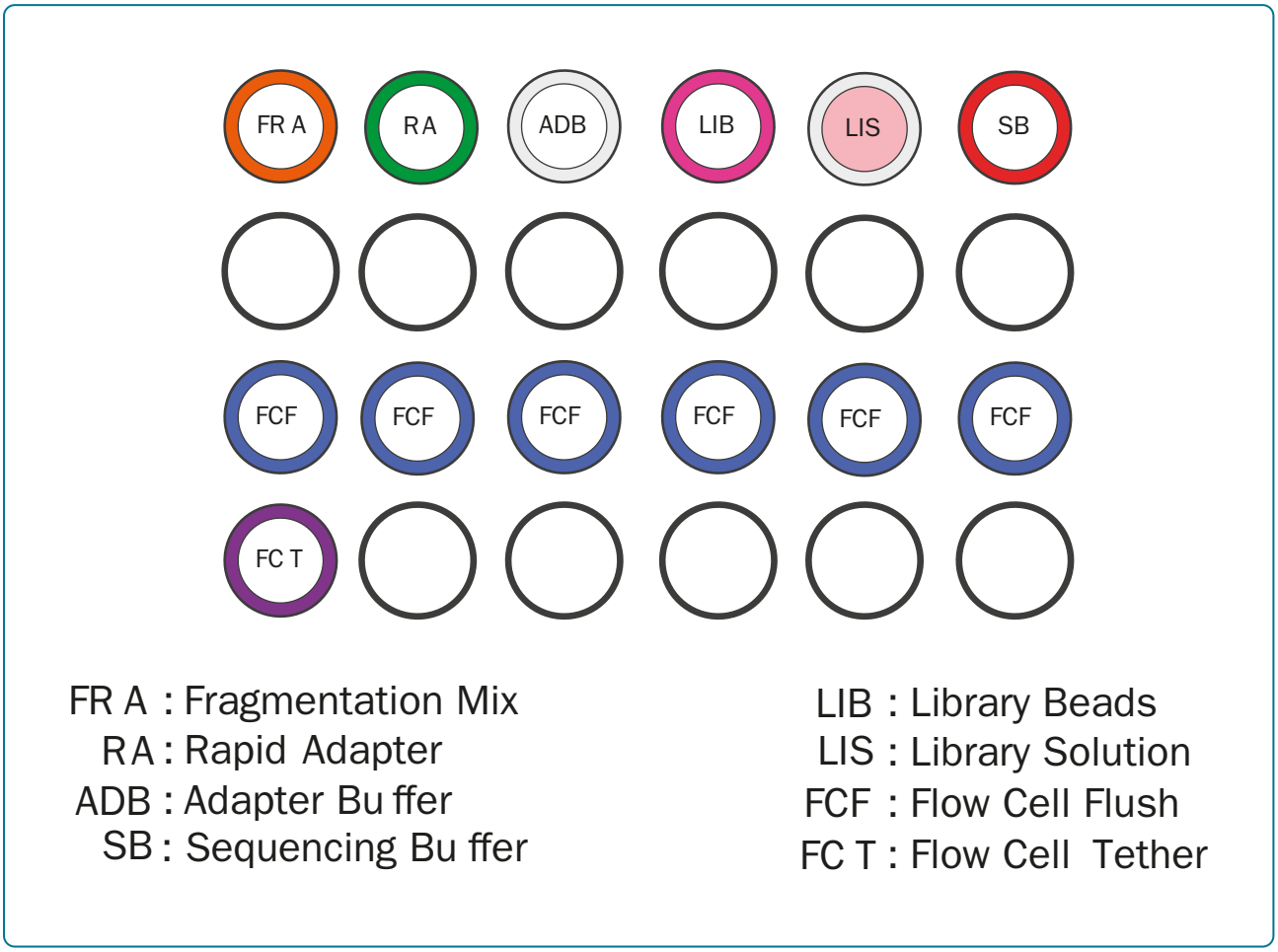
We highly recommend that you check the number of pores in your flow cell prior to starting a sequencing experiment. This should be done within 12 weeks of purchasing for MinION/GridION/PromethION or within four weeks of purchasing Flongle Flow Cells. Oxford Nanopore Technologies will replace any flow cell with fewer than the number of pores in the table below, when the result is reported within two days of performing the flow cell check, and when the storage recommendations have been followed. To do the flow cell check, please follow the instructions in the Flow Cell Check document.

Flow cell	Minimum number of active pores covered by warranty
Flongle Flow Cell	50
MinION/GridION Flow Cell	800
PromethION Flow Cell	5000

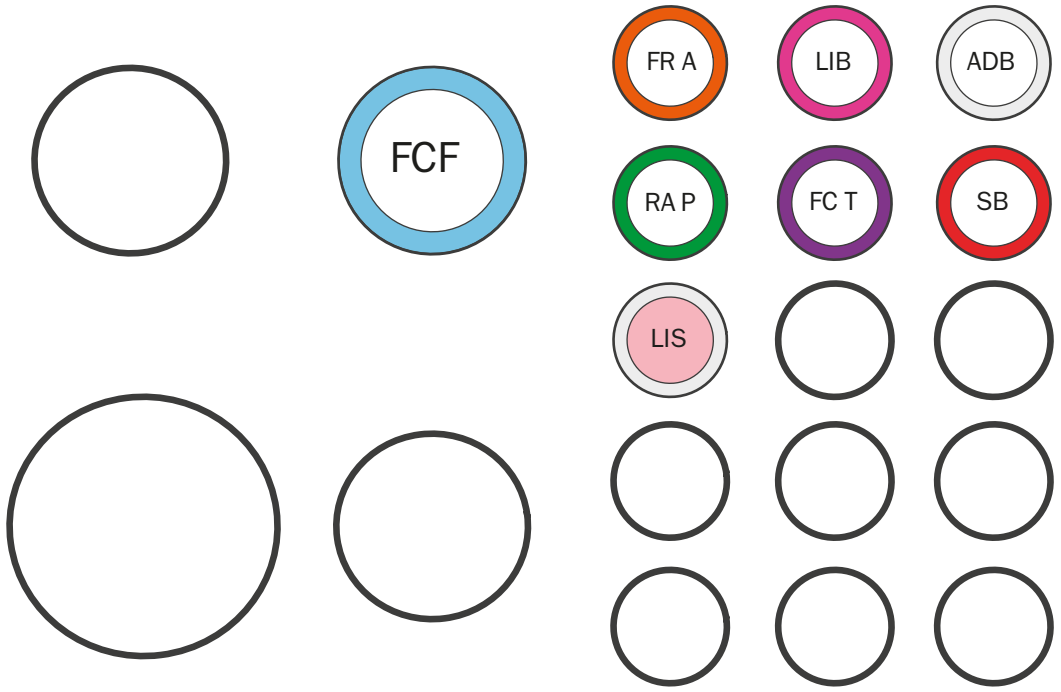
Rapid Sequencing Kit V14 (SQK-RAD114) contents

Note: We are in the process of reformatting our kits with single-use tubes into a bottle format.

Single-use tubes format:



Bottle format:



FR A : Fragmentation Mix
RA : Rapid Adapter
ADB : Adapter Buffer
SB : Sequencing Buffer

LIB : Library Beads
LIS : Library Solution
FCF : Flow Cell Flush
FC T : Flow Cell Tether

3. Library preparation

Materials

100-150 ng high molecular weight genomic DNA
Rapid Adapter (RA)
Adapter Buffer (ADB)
Fragmentation Mix (FRA)

Consumables

Nuclease-free water (e.g. ThermoFisher, AM9937)
1.5 ml Eppendorf DNA LoBind tubes
0.2 ml thin-walled PCR tubes

Equipment

Thermal cycler or heat blocks
P2 pipette and tips
P10 pipette and tips

CHECKPOINT

Check your flow cell.

We recommend performing a flow cell check before starting your library prep to ensure you have a flow cell with enough pores for a good sequencing run.

See the flow cell check instructions in the MinKNOW protocol for more information.

Thaw the kit components at room temperature, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:

Reagent	1. Thaw at room temperature	2. Briefly spin down	3. Mix well by pipetting
Fragmentation Mix (FRA)	Not frozen	✓	✓
Rapid Adapter (RA)	Not frozen	✓	✓
Adapter Buffer (ADB)	Not frozen	✓	✓

1 Once thawed, keep all the kit components on ice.

2 Prepare the DNA in nuclease-free water.

- Transfer 100-150 ng genomic DNA into a 1.5 ml Eppendorf DNA LoBind tube
- Adjust the volume to 10 µl with nuclease-free water
- Mix by flicking the tube to avoid unwanted shearing
- Spin down briefly in a microfuge

3 In a 0.2 ml thin-walled PCR tube, mix the following:

Reagent	Volume
100-150 ng template DNA	10 µl
Fragmentation Mix (FRA)	1 µl
Total	**11 µl**

4. **Mix gently by flicking the tube, and spin down.**

5. **Incubate the tube at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tube on ice to cool it down.**

> **CHECKPOINT**
>
> **The tagmented DNA in 11 µl is taken into the adapter attachment step.**

6. **In a fresh 1.5 ml Eppendorf DNA LoBind tube, dilute the Rapid Adapter (RA) as follows and pipette mix:**

Reagents	Volume
Rapid Adapter (RA)	1.5 µl
Adapter Buffer (ADB)	3.5 µl
Total	**5 µl**

7. **Add 1 µl of diluted Rapid Adapter (RA) to the tagmented DNA.**

8. **Mix gently by flicking the tube, and spin down.**

9. **Incubate the reaction for 5 minutes at room temperature.**

END OF STEP

The prepared DNA library is used for loading into the flow cell. Store the library on ice until ready to load.

4. Priming and loading the MinION and GridION Flow Cell

Materials

Flow Cell Flush (FCF)
Flow Cell Tether (FCT)
Library Solution (LIS)
Library Beads (LIB)
Sequencing Buffer (SB)

Consumables

MinION and GridION Flow Cell
Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)
Nuclease-free water (e.g. ThermoFisher, AM9937)
1.5 ml Eppendorf DNA LoBind tubes

Equipment

MinION or GridION device
MinION and GridION Flow Cell Light Shield
P1000 pipette and tips
P100 pipette and tips
P20 pipette and tips
P10 pipette and tips

IMPORTANT

Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).

TIP

Priming and loading a flow cell

We recommend all new users watch the 'Priming and loading your flow cell' video before your first run.

1 Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at room temperature before mixing by vortexing. Then spin down and store on ice.

IMPORTANT

For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.

Note: We do not recommend using any other albumin type (e.g. recombinant human serum albumin).

2 To prepare the flow cell priming mix with BSA, combine Flow Cell Flush (FCF) and Flow Cell Tether (FCT), as directed below. Mix by pipetting at room temperature.

Note: We are in the process of reformatting our kits with single-use tubes into a bottle format. Please follow the instructions for your kit format.

Single-use tubes format: Add 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml and 30 µl Flow Cell Tether (FCT) directly to a tube of Flow Cell Flush (FCF).

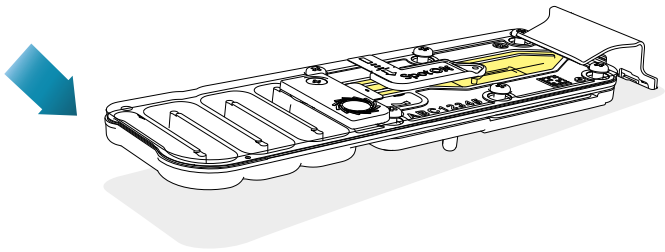
Bottle format: In a suitable tube for the number of flow cells, combine the following reagents:

Reagent	Volume per flow cell
Flow Cell Flush (FCF)	1,170 µl
Bovine Serum Albumin (BSA) at 50 mg/ml	5 µl
Flow Cell Tether (FCT)	30 µl
Total volume	**1,205 µl**

3 Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the priming port cover to ensure correct thermal and electrical contact.

1a

Insert the flow cell into the device under the clip and press down firmly.

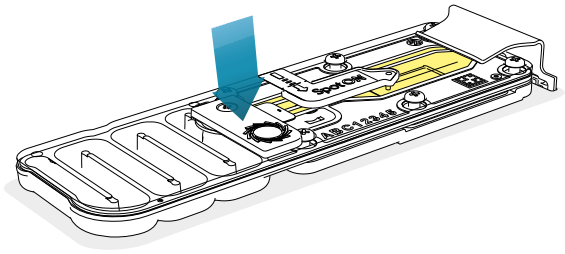


Key:

- Storage buffer
- Priming mix
- DNA library

1b

Insert the flow cell into the device under the clip and press down firmly on the Priming port cover.



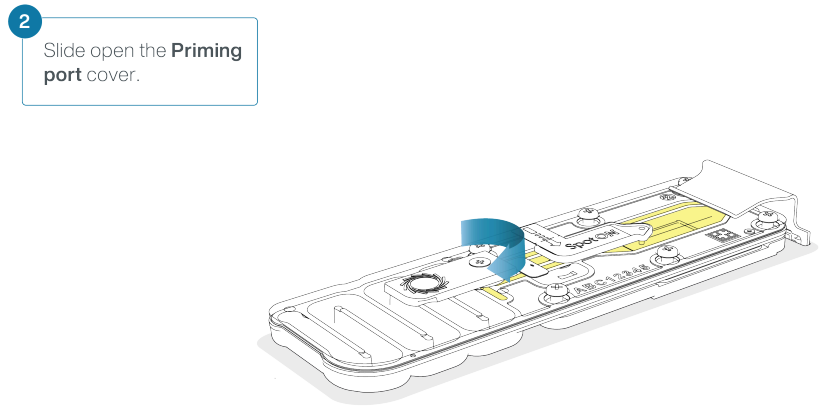
OPTIONAL ACTION

Complete a flow cell check to assess the number of pores available before loading the library.

This step can be omitted if the flow cell has been checked previously.

See the flow cell check instructions in the MinKNOW protocol for more information.

4 Slide the flow cell priming port cover clockwise to open the priming port.



IMPORTANT

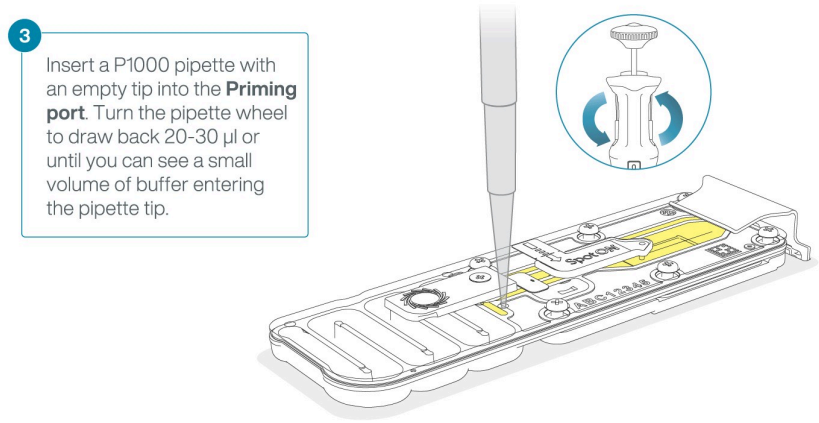
Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

5 After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

1. Set a P1000 pipette to 200 µl

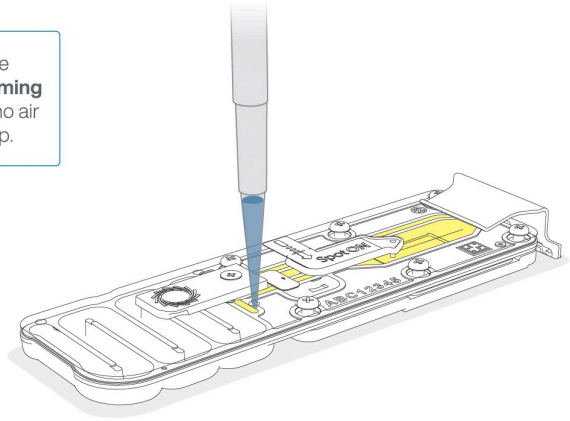
2. Insert the tip into the priming port
3. Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip

Note: Visually check that there is continuous buffer from the priming port across the sensor array.



6 **Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. During this time, prepare the library for loading by following the steps below.**

4 Slowly load 800 µl of the priming mix into the **Priming port**. Ensure there are no air bubbles in the pipette tip.



Wait 5 minutes before proceeding to the next step.

IMPORTANT

The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

We recommend using the Library Beads (LIB) for most sequencing experiments. However, the Library Solution (LIS) is available for more viscous libraries.

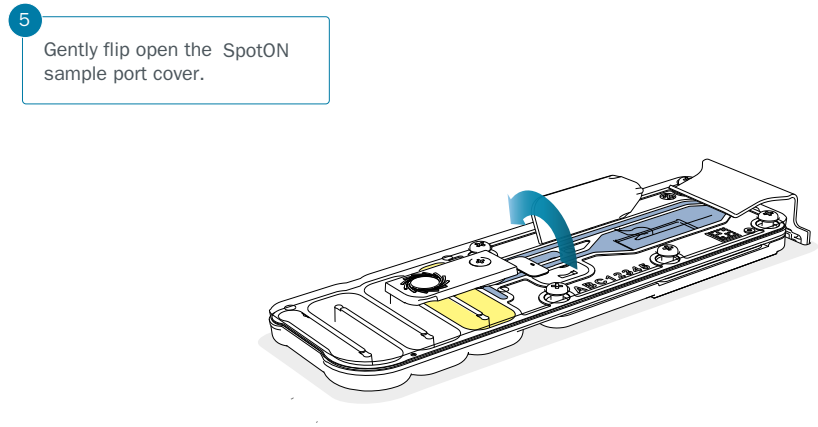
7 Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

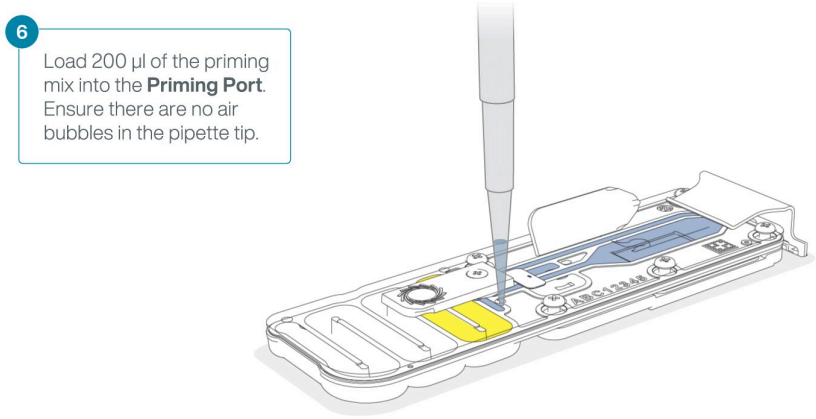
8 In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

Reagent	Volume per flow cell
Sequencing Buffer (SB)	37.5 µl
Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using	25.5 µl
DNA library	12 µl
Total	**75 µl**

9 Complete the flow cell priming:

1. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
2. Load **200 µl** of the priming mix into the flow cell priming port (**not** the SpotON sample port), avoiding the introduction of air bubbles.

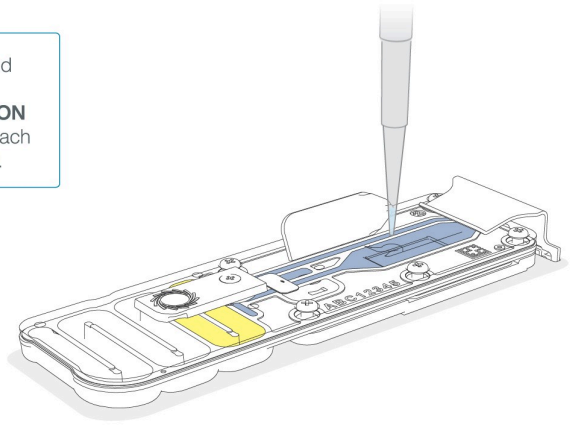




10 **Mix the prepared library gently by pipetting up and down just prior to loading.**

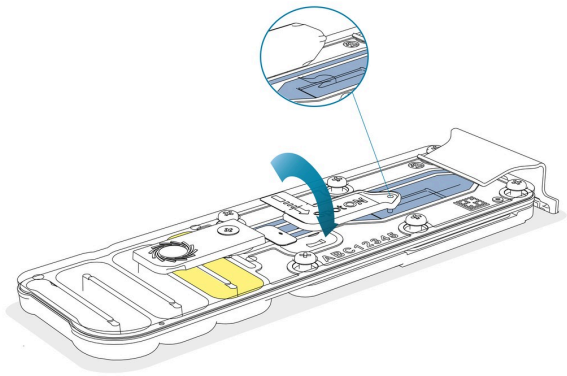
11 **Add 75 µl of the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.**

7 Pipette mix the prepared library and load 75 µl dropwise into the **SpotON** sample port, ensuring each drop flows into the port.

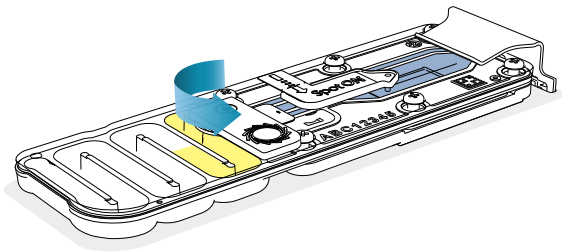


12 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.

8 Gently replace the **SpotON** sample port cover.



9 Gently close the **Priming port**.



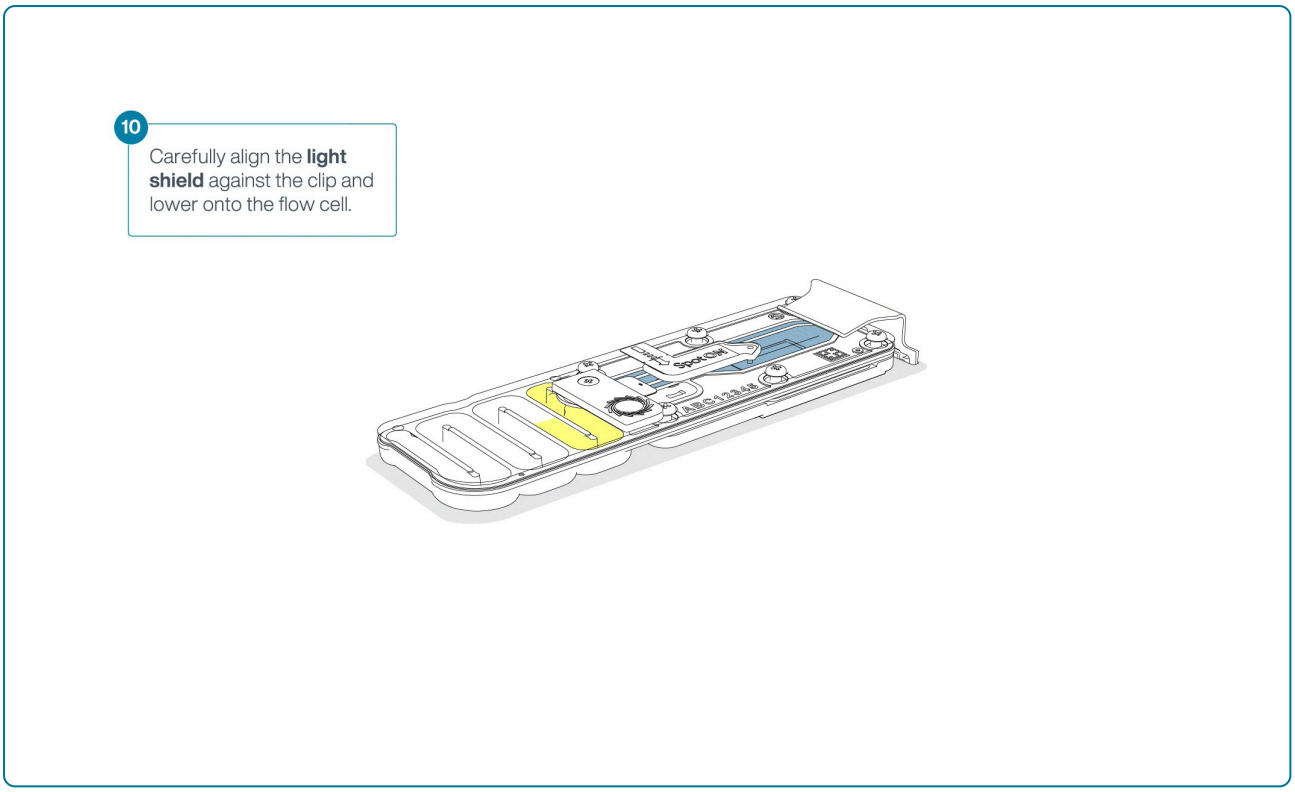
IMPORTANT

Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.

We recommend leaving the light shield on the flow cell when library is loaded, including during any washing and reloading steps. The shield can be removed when the library has been removed from the flow cell.

13 Place the light shield onto the flow cell, as follows:

1. Carefully place the leading edge of the light shield against the clip. **Note:** Do not force the light shield underneath the clip.
2. Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.



CAUTION

The MinION Flow Cell Light Shield is not secured to the flow cell and careful handling is required after installation.

END OF STEP

Close the device lid and set up a sequencing run on MinKNOW.

5. Data acquisition and basecalling

How to start sequencing

Once you have loaded your flow cell, the sequencing run can be started on MinKNOW, our sequencing software that controls the device, data acquisition and real-time basecalling. For more detailed information on setting up and using MinKNOW, please see the MinKNOW protocol.

MinKNOW can be used and set up to sequence in multiple ways:

- On a computer either directly or remotely connected to a sequencing device.
- Directly on a GridION or PromethION 24/48 sequencing device.

For more information on using MinKNOW on a sequencing device, please see the device user manuals:

- MinION Mk1B user manual
- MinION Mk1C user manual
- MinION Mk1D user manual
- GridION user manual

To start a sequencing run on MinKNOW:

1. Navigate to the start page and click **Start sequencing.**

2. Fill in your experiment details, such as name and flow cell position and sample ID.

3. Select the **sequencing kit used in the library preparation** on the Kit page.

4. Configure the sequencing and output parameters for your sequencing run or keep to the default settings on the Run configuration tab.

Note: If basecalling was turned off when a sequencing run was set up, basecalling can be performed post-run on MinKNOW. For more information, please see the MinKNOW protocol.

5. Click **Start** to initiate the sequencing run.

Data analysis after sequencing

After sequencing has completed on MinKNOW, the flow cell can be reused or returned, as outlined in the Flow cell reuse and returns section.

After sequencing and basecalling, the data can be analysed. For further information about options for basecalling and post-basecalling analysis, please refer to the Data Analysis document.

In the Downstream analysis section, we outline further options for analysing your data.

6. Flow cell reuse and returns

Materials Flow Cell Wash Kit (EXP-WSH004)

1 **After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Flow Cell Wash Kit protocol and store the washed flow cell at +2°C to +8°C.**

The Flow Cell Wash Kit protocol is available on the Nanopore Community.

TIP

We recommend you to wash the flow cell as soon as possible after you stop the run. However, if this is not possible, leave the flow cell on the device and wash it the next day.

2 **Alternatively, follow the returns procedure to send the flow cell back to Oxford Nanopore.**

Instructions for returning flow cells can be found here.

> **IMPORTANT**
>
> **If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in the online version of this protocol.**

7. Downstream analysis

Post-basecalling analysis

There are several options for further analysing your basecalled data:

1. EPI2ME workflows

For in-depth data analysis, Oxford Nanopore Technologies offers a range of bioinformatics tutorials and workflows available in EPI2ME. The platform provides a vehicle where workflows deposited in GitHub by our Research and Applications teams can be showcased with descriptive texts, functional bioinformatics code and example data.

2. Research analysis tools

Oxford Nanopore Technologies' Research division has created a number of analysis tools, which are available in the Oxford Nanopore GitHub repository. The tools are aimed at advanced users, and contain instructions for how to install and run the software. They are provided as-is, with minimal support.

3. Community-developed analysis tools

If a data analysis method for your research question is not provided in any of the resources above, please refer to the resource centre and search for bioinformatics tools for your application. Numerous members of the Nanopore Community have developed their own tools and pipelines for analysing nanopore sequencing data, most of which are available on GitHub. Please be aware that these tools are not supported by Oxford Nanopore Technologies, and are not guaranteed to be compatible with the latest chemistry/software configuration.

8. Issues during DNA/RNA extraction and library preparation

Below is a list of the most commonly encountered issues, with some suggested causes and solutions.

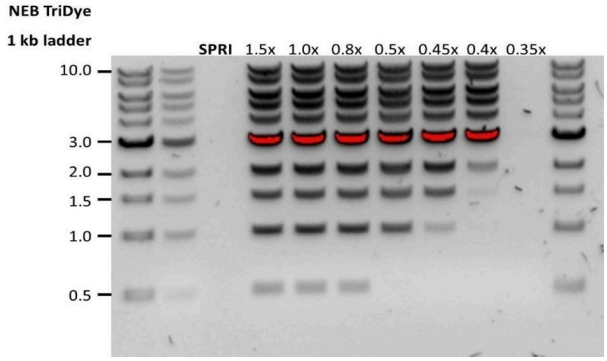
We also have an FAQ section available on the Nanopore Community Support section.

If you have tried our suggested solutions and the issue still persists, please contact Technical Support via email (support@nanoporetech.com) or via LiveChat in the Nanopore Community.

Low sample quality

Observation	Possible cause	Comments and actions
Low DNA purity (Nanodrop reading for DNA OD 260/280 is <1.8 and OD 260/230 is <2.0–2.2)	The DNA extraction method does not provide the required purity	The effects of contaminants are shown in the Contaminants document. Please try an alternative extraction method that does not result in contaminant carryover. Consider performing an additional SPRI clean-up step.
Low RNA integrity (RNA integrity number <9.5 RIN, or the rRNA band is shown as a smear on the gel)	The RNA degraded during extraction	Try a different RNA extraction method. For more info on RIN, please see the RNA Integrity Number document. Further information can be found in the DNA/RNA Handling page.
RNA has a shorter than expected fragment length	The RNA degraded during extraction	Try a different RNA extraction method. For more info on RIN, please see the RNA Integrity Number document. Further information can be found in the DNA/RNA Handling page. We recommend working in an RNase-free environment, and to keep your lab equipment RNase-free when working with RNA.

Low DNA recovery after AMPure bead clean-up

Observation	Possible cause	Comments and actions
Low recovery	DNA loss due to a lower than intended AMPure beads-to-sample ratio	1. AMPure beads settle quickly, so ensure they are well resuspended before adding them to the sample. 2. When the AMPure beads-to-sample ratio is lower than 0.4:1, DNA fragments of any size will be lost during the clean-up.
Low recovery	DNA fragments are shorter than expected	The lower the AMPure beads-to-sample ratio, the more stringent the selection against short fragments. Please always determine the input DNA length on an agarose gel (or other gel electrophoresis methods) and then calculate the appropriate amount of AMPure beads to use. 
Low recovery after end-prep	The wash step used ethanol <70%	DNA will be eluted from the beads when using ethanol <70%. Make sure to use the correct percentage.

9. Issues during the sequencing run using a Rapid-based sequencing kit

Below is a list of the most commonly encountered issues, with some suggested causes and solutions.

We also have an FAQ section available on the Nanopore Community Support section.

If you have tried our suggested solutions and the issue still persists, please contact Technical Support via email (support@nanoporetech.com) or via LiveChat in the Nanopore Community.

Fewer pores at the start of sequencing than after Flow Cell Check

Observation	Possible cause	Comments and actions
MinKNOW reported a lower number of pores at the start of sequencing than the number reported by the Flow Cell Check	An air bubble was introduced into the nanopore array	After the Flow Cell Check it is essential to remove any air bubbles near the priming port before priming the flow cell. If not removed, the air bubble can travel to the nanopore array and irreversibly damage the nanopores that have been exposed to air. The best practice to prevent this from happening is demonstrated in this video.
MinKNOW reported a lower number of pores at the start of sequencing than the number reported by the Flow Cell Check	The flow cell is not correctly inserted into the device	Stop the sequencing run, remove the flow cell from the sequencing device and insert it again, checking that the flow cell is firmly seated in the device and that it has reached the target temperature. If applicable, try a different position on the device (GridION/PromethION).
MinKNOW reported a lower number of pores at the start of sequencing than the number reported by the Flow Cell Check	Contaminations in the library damaged or blocked the pores	The pore count during the Flow Cell Check is performed using the QC DNA molecules present in the flow cell storage buffer. At the start of sequencing, the library itself is used to estimate the number of active pores. Because of this, variability of about 10% in the number of pores is expected. A significantly lower pore count reported at the start of sequencing can be due to contaminants in the library that have damaged the membranes or blocked the pores. Alternative DNA/RNA extraction or purification methods may be needed to improve the purity of the input material. The effects of contaminants are shown in the Contaminants Know-how piece. Please try an alternative extraction method that does not result in contaminant carryover.

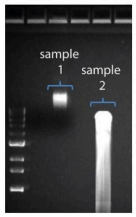
MinKNOW script failed

Observation	Possible cause	Comments and actions
MinKNOW shows "Script failed"		Restart the computer and then restart MinKNOW. If the issue persists, please collect the MinKNOW log files and contact Technical Support. If you do not have another sequencing device available, we recommend storing the flow cell and the loaded library at 4°C and contact Technical Support for further storage guidance.

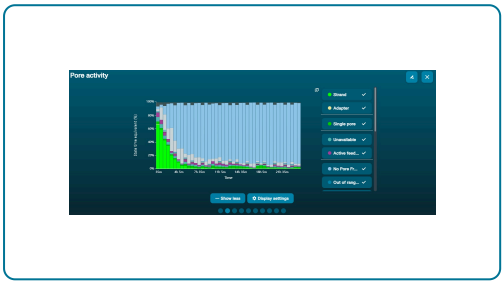
Pore occupancy below 40%

Observation	Possible cause	Comments and actions
Pore occupancy <40%	Not enough library was loaded on the flow cell	Ensure the correct concentration of good quality library is loaded on to a MinION/GridION flow cell. To check the concentration, please refer to the library preparation protocol. Please quantify the library before loading and calculate mols using tools like the Promega Biomath Calculator, choosing "dsDNA: µg to pmol"
Pore occupancy close to 0	The Rapid Sequencing Kit V14/Rapid Barcoding Kit V14 was used, and sequencing adapters did not attach to the DNA	Make sure to closely follow the protocol and use the correct volumes and incubation temperatures. A Lambda control library can be prepared to test the integrity of reagents.
Pore occupancy close to 0	No tether on the flow cell	Tethers are adding during flow cell priming (FCT tube). Make sure FCT was added to FCF before priming.

Shorter than expected read length

Observation	Possible cause	Comments and actions
Shorter than expected read length	Unwanted fragmentation of DNA sample	Read length reflects input DNA fragment length. Input DNA can be fragmented during extraction and library prep. 1. Please review the Extraction Methods in the Nanopore Community for best practice for extraction. 2. Visualise the input DNA fragment length distribution on an agarose gel before proceeding to the library prep.  In the image above, Sample 1 is of high molecular weight, whereas Sample 2 has been fragmented. 3. During library prep, avoid pipetting and vortexing when mixing reagents. Flicking or inverting the tube is sufficient.

Large proportion of unavailable pores

Observation	Possible cause	Comments and actions
Large proportion of unavailable pores (shown as blue in the channels panel and pore activity plot)  The pore activity plot above shows an increasing proportion of "unavailable" pores over time.	Contaminants are present in the sample	Some contaminants can be cleared from the pores by the unblocking function built into MinKNOW. If this is successful, the pore status will change to "sequencing pore". If the portion of unavailable pores stays large or increases: 1. A nuclease flush using the Flow Cell Wash Kit (EXP-WSH004) can be performed, or 2. Run several cycles of PCR to try and dilute any contaminants that may be causing problems.

Large proportion of inactive pores

Observation	Possible cause	Comments and actions
Large proportion of inactive/unavailable pores (shown as light blue in the channels panel and pore activity plot. Pores or membranes are irreversibly damaged)	Air bubbles have been introduced into the flow cell	Air bubbles introduced through flow cell priming and library loading can irreversibly damage the pores. Watch the Priming and loading your flow cell video for best practice
Large proportion of inactive/unavailable pores	Certain compounds co-purified with DNA	Known compounds, include polysaccharides, typically associate with plant genomic DNA. 1. Please refer to the Plant leaf DNA extraction method. 2. Clean-up using the QIAGEN PowerClean Pro kit. 3. Perform a whole genome amplification with the original gDNA sample using the QIAGEN REPLI-g kit.
Large proportion of inactive/unavailable pores	Contaminants are present in the sample	The effects of contaminants are shown in the Contaminants Know-how piece. Please try an alternative extraction method that does not result in contaminant carryover.

Temperature fluctuation

Observation	Possible cause	Comments and actions
Temperature fluctuation	The flow cell has lost contact with the device	Check that there is a heat pad covering the metal plate on the back of the flow cell. Re-insert the flow cell and press it down to make sure the connector pins are firmly in contact with the device. If the problem persists, please contact Technical Services.

Failed to reach target temperature

Observation	Possible cause	Comments and actions
MinKNOW shows "Failed to reach target temperature"	The instrument was placed in a location that is colder than normal room temperature, or a location with poor ventilation (which leads to the flow cells overheating)	MinKNOW has a default timeframe for the flow cell to reach the target temperature. Once the timeframe is exceeded, an error message will appear and the sequencing experiment will continue. However, sequencing at an incorrect temperature may lead to a decrease in throughput and lower q-scores. Please adjust the location of the sequencing device to ensure that it is placed at room temperature with good ventilation, then re-start the process in MinKNOW. Please refer to this link for more information on MinION temperature control.