PROTOCOL

Flow Cell Wash Kit (EXP-WSH004 or EXP-WSH004-XL) VWFC_9120_v1_revQ_08Dec2020

For Research Use Only

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1. Overview of the protocol

Introduction to the Flow Cell Wash Kit

The Flow Cell Wash Kit allows sequential runs of multiple sequencing libraries on the same flow cell. It works by flushing out and digesting the first library, refreshing the system for a subsequent library to be loaded. This procedure provides the opportunity to utilise the same flow cell a number of times, maximising the available run time, particularly for cases where less data per library is required. Following the wash step, Storage Buffer can be introduced into the flow cell, allowing storage of the flow cell at 4-8°C. The Flow Cell Wash Kit is compatible with Q-Line, R9.4.1 and R10.4.1 flow cells.

The nuclease digests DNA library and for effective removal from the flow cell before loading a new library, we recommend removing the waste buffer from the waste ports after each flush step. This is to ensure there is no nuclease in the waste channel that may diffuse through the flow cell during sequencing. To further improve flow cell output after a wash, we recommend pipetting slowly during flushing steps by either twisting the pipette wheel down slowly or pressing the plunger down slowly.

Please note, although the wash procedure should remove 99.9% of the library, some residual DNA may remain on the flow cell. For this reason, we recommend to barcode your libraries when used in conjunction with the Flow Cell Wash Kit, to ensure that reads from different libraries can be separated from each other.

Successful deconvolution of DNA reads has been demonstrated in Oxford Nanopore's internal development:

Figure 1. A sample with four barcodes was sequenced and washed using EXP-WSH004 before a sample with four different barcodes was loaded. This was repeated for a third sequencing run.

For users who wish to use barcoding to run multiple libraries at one time rather than washing the flow cells, please see the barcoding kits we have available in the chemistry technical document.

The Flow Cell Wash Kit is can also be used with our RNA flow cells to flush out RNA libraries, but will not remove RNA-related blocking and restore nanopores.

Nuclease activity of the Flow Cell Wash Kit

The Flow Cell Wash Kit contains DNase I, that is used to digest any remaining DNA library on a flow cell. Once the library is removed, the flow cell can be re-used immediately or stored for later use.

During sequencing, an accumulation of pores in the “unavailable" state (Figure 2) may be observed, causing the rate of data acquisition to decline as fewer pores are available to accept and sequence strands. We have demonstrated that in these circumstances, pores can be reverted to the “active pore” state by pausing sequencing and washing the flow cell with the DNase I in the Flow Cell Wash Kit. In Figure 2, the astrisks indicate where sequencing has been paused and the flow cell washed. **Note:** If the sequencing run is paused in MinKNOW for the flow cell wash, you will only see the restoration of sequencing pores after a new pore scan has been performed.

The wash step is recommended where sequencing channels are lost to the “unavailable” state (Figure 2). In circumstances where channels have been lost by other means, for example “saturated”, the wash step is not effective at reverting channels to the “active pore” state. If your sample is not known to block and cause saturation of the flow cell, we recommend loading a fresh library on a new flow cell. If you do not have excess library to load on a new flow cell, you can recover the library and reload on a new flow cell following method 1 of the Library recovery from flow cells protocol.

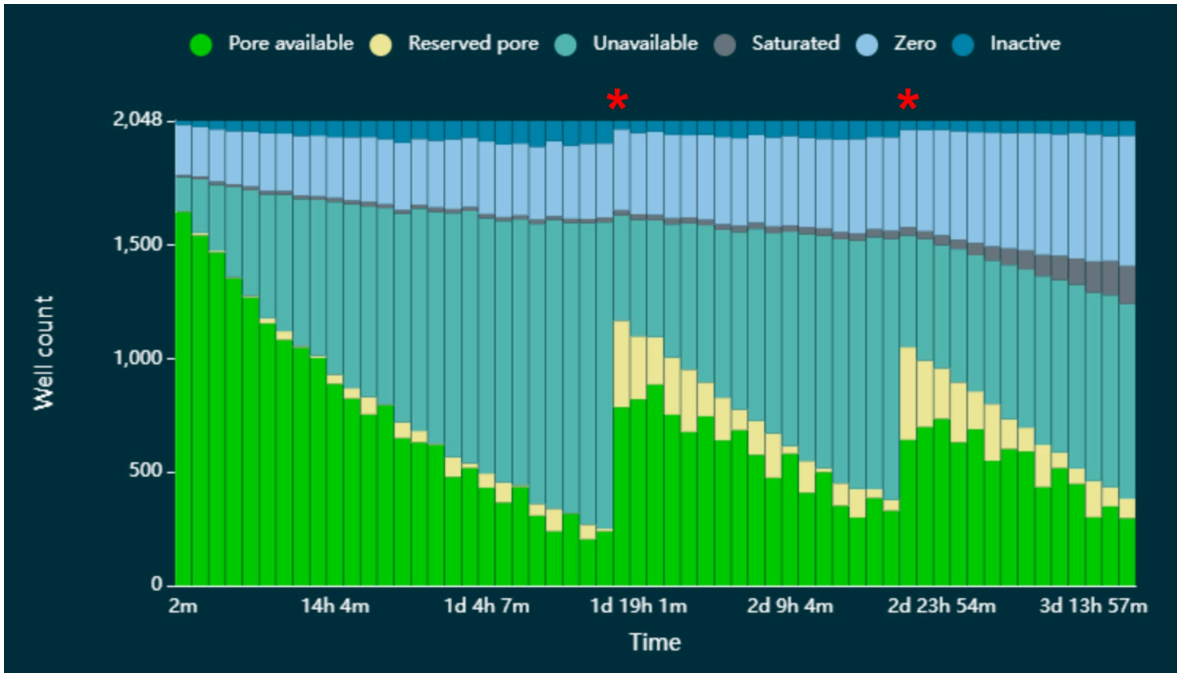


Figure 2. Pore states observed on a flow cell before and after wash steps are performed. A flow cell has been loaded with a sequencing library that has resulted in an accumulation of pores in the “unavailable” state, leading to a decrease in the rate of data acquisition. The red asterisks indicate when a wash step has been performed. A significant number of the pores that had been lost to the “unavailable” state have reverted to the “Pore available” state and are available for sequencing once again.

In experiments where output is limited by the increase in pores in the “unavailable” state, we have shown that output can be improved by performing several wash steps over the lifetime of a flow cell. Figure 3 shows the output obtained from a PromethION Flow Cell loaded with a library of DNA extracted from chicken - *Gallus gallus*, and a MinION Flow Cell loaded with a library of DNA extracted

from a type of Japense ricefish - *Oryzias latipes*, where unavailable pores increased over the course of the experiment, and so flow cell washes were performed to unblock the pores (Figure 3). In each case, the use of multiple washes allowed for an improvement of the output from the flow cell, without any compromise in observed read length (Figure 4).

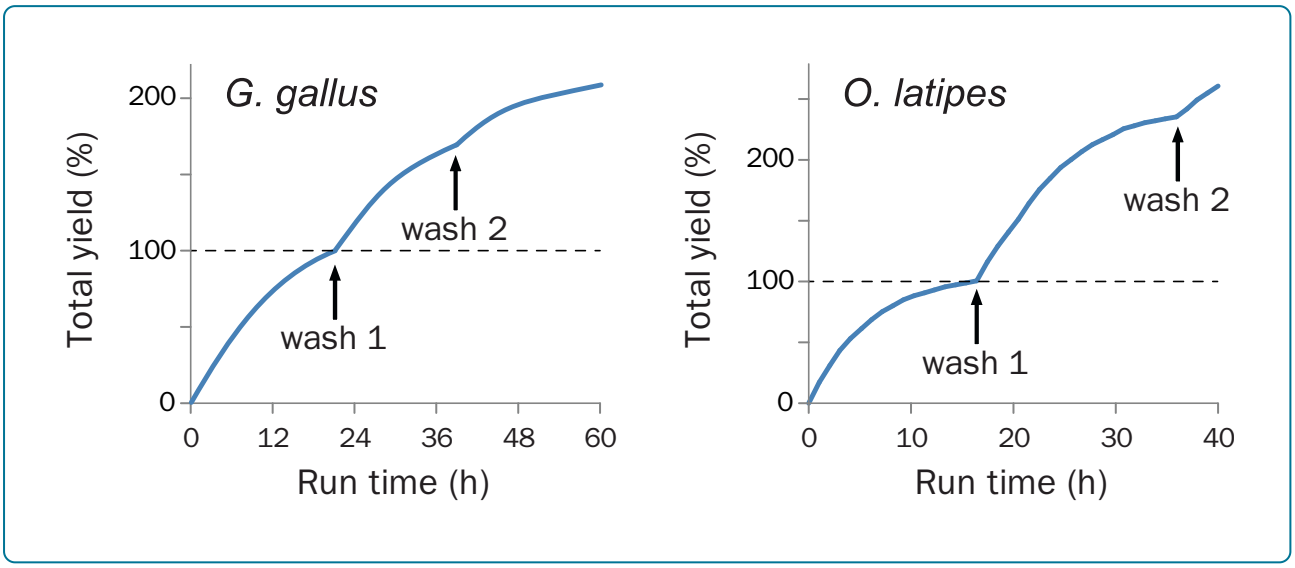


Figure 3. Throughput observed from *Gallus gallus* and *Oryzias latipes* libraries run on a PromethION Flow Cell and a MinION Flow Cell, respectively. The arrows indicate the timing of each wash step: wash steps were performed at the point where the rate of data acquisition started to slow due to the accumulation of "recovering" pores. In each case, output is more than doubled from the point of the first wash.

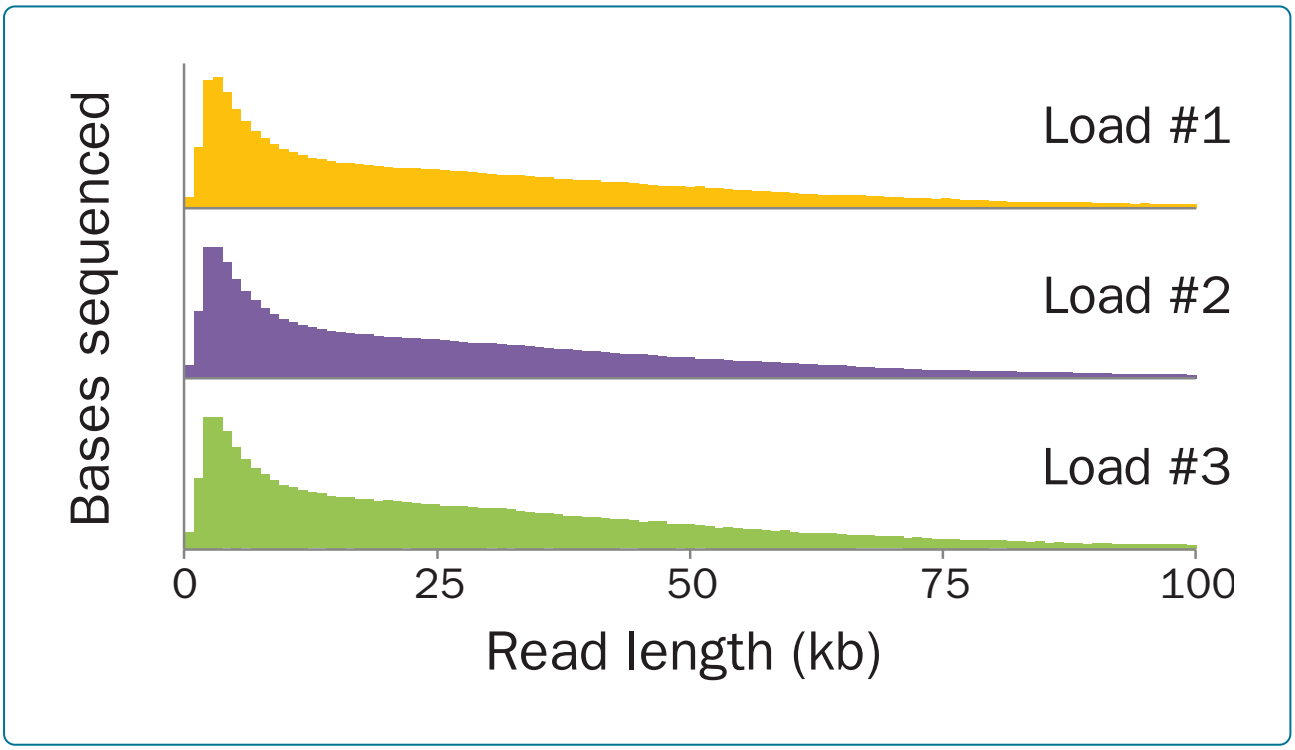


Figure 4. Effective inactivation of the DNase I prevents read length deterioration in the experiments after a nuclease wash is performed. In this example, the read length of the *Gallus gallus* library was recorded before the first wash (load #1) and then again after the first and second washes (load #2 and #3, respectively). No decrease in read length is observed.

2. Equipment and consumables

Materials

Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)
Flow cell priming reagents available in your sequencing kit or in the following kits:
Sequencing Auxiliary Vials V14 (EXP-AUX003)
Flow Cell Priming Kit (EXP-FLP004)

Equipment

P1000 pipette and tips
P20 pipette and tips
Ice bucket with ice

Reloading a library

Additional buffers are required for reloading a library following the washing of a flow cell. These can be found in the one of the following expansion kits:

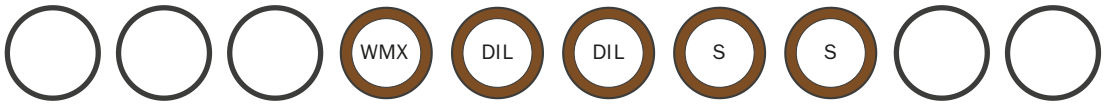
- Sequencing Auxiliary Vials V14 (EXP-AUX003). This expansion contains vials of Sequencing Buffer (SB), Elution Buffer (EB), Library Solution (LIS), and Library Beads (LIB) with Kit 14 flow cell priming reagents: Flow Cell Flush (FCF) and Flow Cell Tether (FCT). This expansion is only compatible with our Kit 14 chemistry e.g. SQK-LSK114.
- Flow Cell Priming Kit (EXP-FLP004). This expansion contains both Kit 14 flow cell priming reagents required: Flow Cell Flush (FCF) and Flow Cell Tether (FCT). This expansion is only compatible with Kit 14 chemistry.

For our previous chemistries:

- Sequencing Auxiliary Vials expansion (EXP-AUX001). This expansion contains vials of Elution Buffer (EB), Sequencing Buffer (SQB), and Loading Beads (LB), additional to those found in DNA sequencing kits for our Kit 9 chemistry.
- Sequencing Auxiliary Vials expansion (EXP-AUX002). This expansion contains vials of Sequencing Buffer II (SBII), Elution Buffer (EB), Loading Solution (LS), and Loading Beads II (LBII), additional to those found in Kit 10 and 11 chemistry, such as:
    - Kit 10 e.g. SQK-LSK110
    - Kit 11 e.g. SQK-PCS111

- Flow Cell Priming Kit (EXP-FLO002). This expansion contains both flow cell priming reagents required: Flush Buffer (FB) and Flush Tether (FLT). This expansion is compatible with Kit 9, 10 and 11 chemistry.

Flow Cell Wash Kit (EXP-WSH004) contents

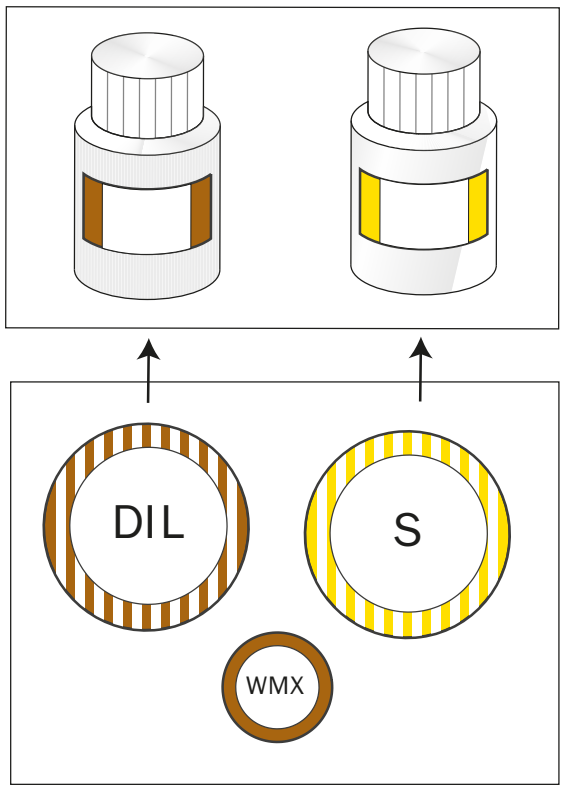


WMX : Wash Mix
DIL : Wash Diluent
S : Storage Buffer

Contents	Volume (µl)	No. of tubes	No. of uses
Wash Mix (WMX)	15	1	6
Wash Diluent (DIL)	1,300	2	6
Storage Buffer (S)	1,600	2	6

- Wash Mix (WMX) contains DNase I.
- Wash Diluent (DIL) contains the exonuclease buffer that maximises activity of the DNase I.
- The Storage Buffer allows flow cells to be stored for extended periods of time.

Flow Cell Wash Kit XL (EXP-WSH004-XL) contents

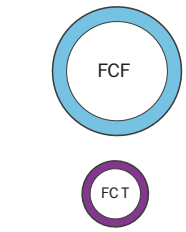


WMX : Wash Mix
DIL : Wash Diluent
S : Storage Bu ffer

Name	Acronym	Fill volume per vial (µl)	Cap colour	No. of vials	No. of uses
Wash Mix	WMX	150	Brown	1	48
Wash Diluent	DIL	20,000	White cap, brown stripe	1	48
Storage Buffer	S	25,000	White cap, yellow stripe	1	48

- Wash Mix (WMX) contains DNase I.
- Wash Diluent (DIL) contains the exonuclease buffer that maximises activity of the DNase I.
- The Storage Buffer allows flow cells to be stored for extended periods of time.

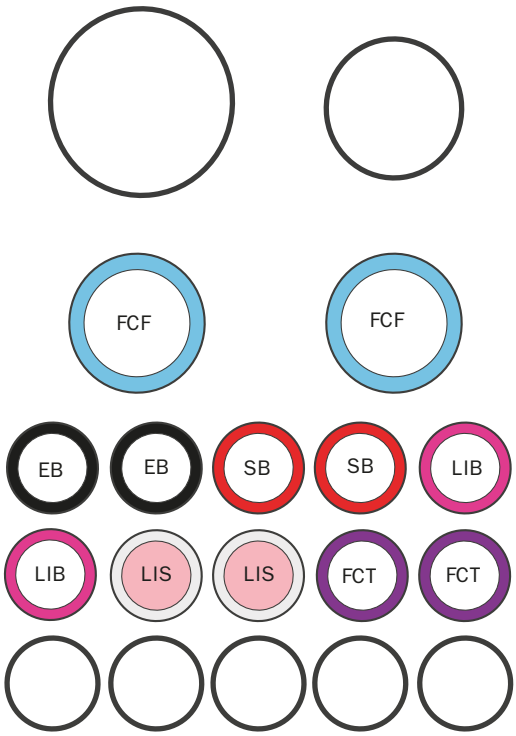
Flow Cell Priming Kit (EXP-FLP004) contents



FCF : Flow Cell Flush
FCT : Flow Cell Tether

Name	Acronym	Cap colour	No. of vials	Fill volume per vial (µl)
Flow Cell Flush	FCF	6	Clear cap, light blue lable	8,000
Flow Cell Tether	FCT	1	Purple	200

Sequencing Auxiliary Vials V14 (EXP-AUX003) contents



EB : Elution Buffer
SB : Sequencing Buffer
LIB : Library Beads
LIS : Library Solution
FCF : Flow Cell Flush
FC T : Flow Cell Tether

Name	Acronym	Cap colour	No. of vials	Fill volume per vial (µl)
Elution Buffer	EB	Black	2	500
Sequencing Buffer	SB	Red	2	700
Library Solution	LIS	White cap, pink label	2	600
Library Beads	LIB	Pink	2	600
Flow Cell Flush	FCF	Light blue label	2	8,000
Flow Cell Tether	FCT	Purple	2	200

3. Flushing a MinION/GridION Flow Cell

Materials Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)

Equipment P1000 pipette and tips
P20 pipette and tips
Ice bucket with ice

Preparation to run the washing procedure

- This protocol assumes that the flow cell has already had a DNA/RNA library run on it
- The aim is to remove most of this initial library from the flow cell
- The Wash Kit contains all solutions required for removal of the initial library
- Data acquisition in MinKNOW should be stopped (if loading a new library or storing the flow cell), or paused (if loading more of the same library after the wash)
- After the flow cell has been washed, a new library can be loaded or the flow cell can be stored at 4°C

TIP

We recommend keeping the light shield on the flow cell during washing if a second library will be loaded straight away.

If the flow cell is to be washed and stored, the light shield can be removed.

IMPORTANT

A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.

1. **Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.**

2. **Thaw one tube of Wash Diluent (DIL) at room temperature.**

3. **Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.**

4. **In a fresh 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:**

Reagent	Volume per flow cell
Wash Mix (WMX)	2 µl
Wash Diluent (DIL)	398 µl
Total	**400 µl**

5 **Mix well by pipetting, and place on ice. Do not vortex the tube.**

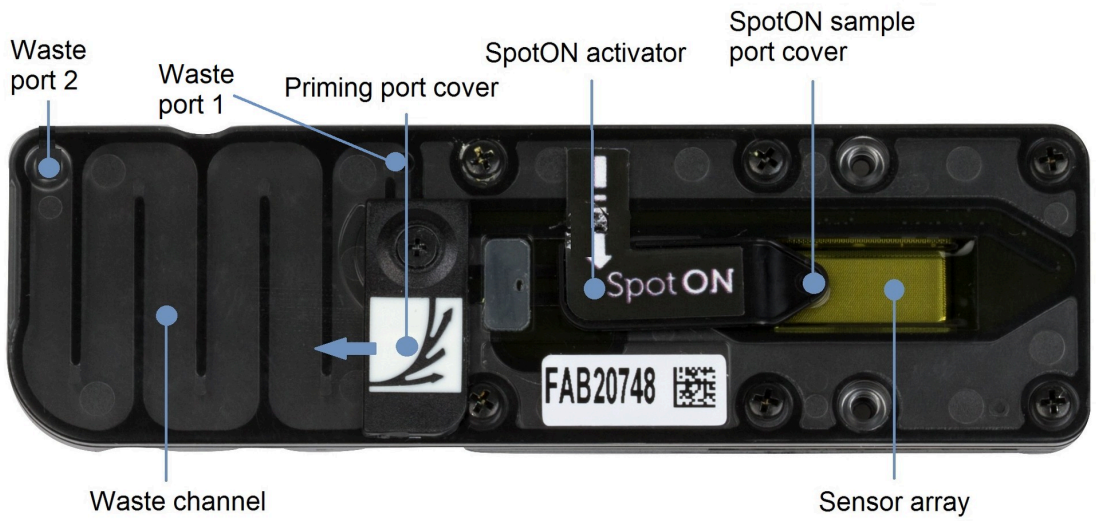
6 **Stop or pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.**

> **IMPORTANT**
>
> **It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.**

7 **Remove the waste buffer, as follows:**

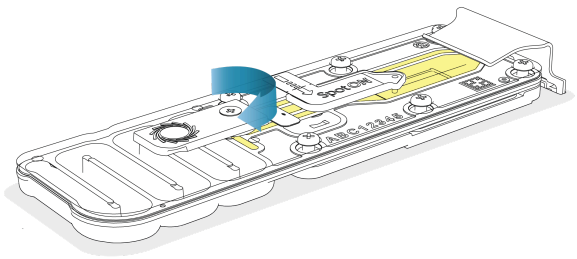
1. Close the priming port and SpotON sample port cover, as indicated in the figure below.
2. Insert a P1000 pipette into waste port 1 and remove the waste buffer.

Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



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

2 Slide open the **Priming port** cover.

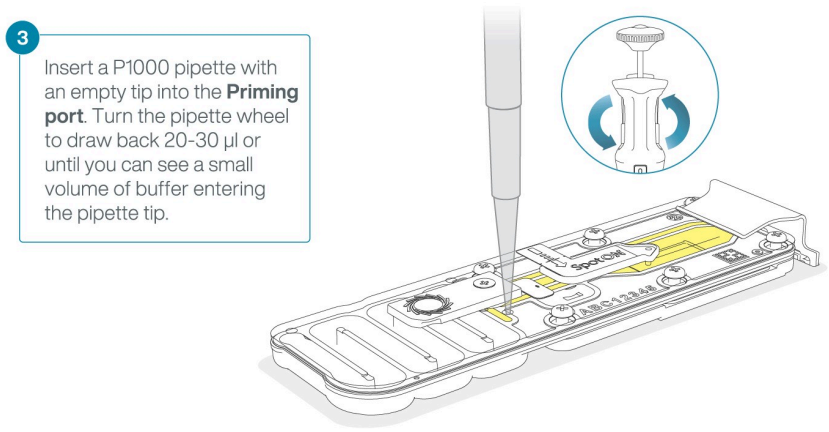


IMPORTANT

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.

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

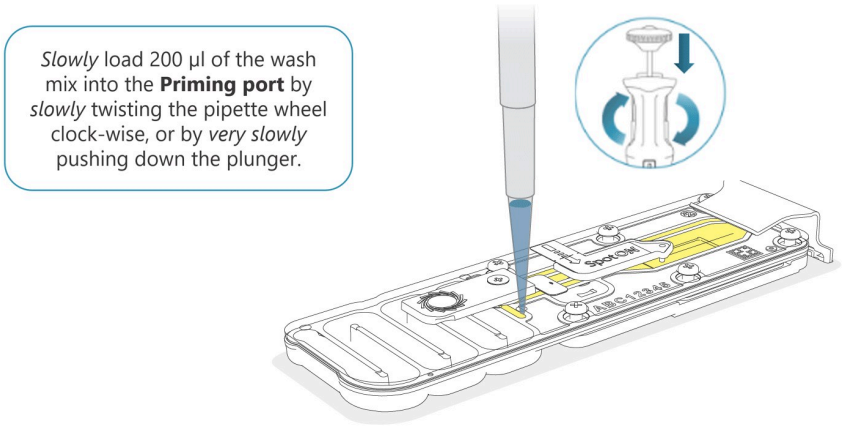
1. Set a P1000 pipette to 200 µl.
2. Insert the tip into the flow cell priming port.
3. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip.
4. Visually check that there is continuous buffer from the flow cell priming port across the sensor array.



10 Slowly load 200 µl of the prepared flow cell wash mix into the priming port, as follows:

1. Using a P1000 pipette, take 200 µl of the flow cell wash mix
2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
3. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, leaving a small volume of buffer in the pipette tip.

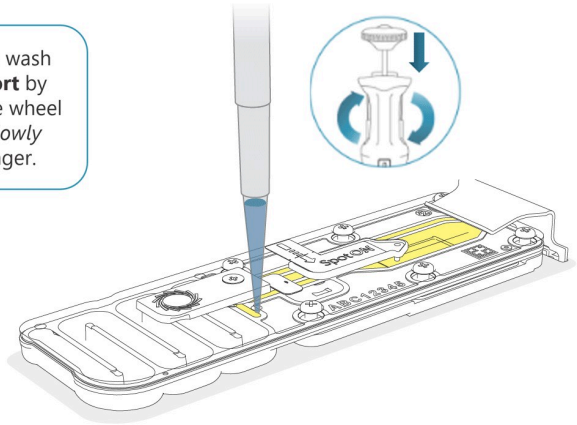
4. Set a timer for a 5 minute incubation.



11 Once the 5 minute incubation is complete, carefully load the remaining 200 µl of the prepared flow cell wash mix into the priming port, as follows:

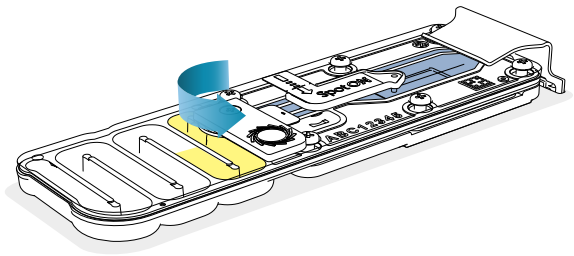
1. Using a P1000 pipette, take the remaining 200 µl of the flow cell wash mix
2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
3. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, leaving a small volume of buffer in the pipette tip.

Slowly load 200 µl of the wash mix into the **Priming port** by *slowly* twisting the pipette wheel clock-wise, or by *very slowly* pushing down the plunger.



12 Close the priming port and wait for 1 hour.

9 Gently close the **Priming port**.



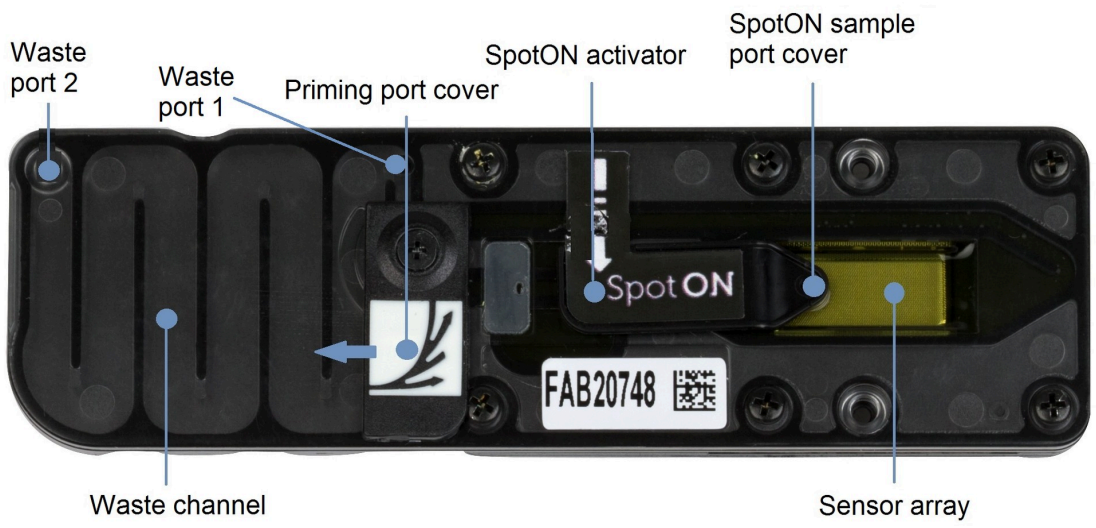
IMPORTANT

It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

13 Remove the waste buffer, as follows:

1. Ensure the priming port and SpotON sample port covers are closed, as indicated in the figure below.
2. Insert a P1000 pipette into waste port 1 and remove the waste buffer.

Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



END OF STEP

Follow one of the two options described in the next steps of the protocol.

- Run a second library on the flow cell straight away
- Store the flow cell for later use

4. To run a second library on a MinION/GridION Flow Cell straight away

Materials

Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)
Flow cell priming reagents available in your sequencing kit or in the following kits:
Sequencing Auxiliary Vials V14 (EXP-AUX003)
Flow Cell Priming Kit (EXP-FLP004)

Equipment

P1000 pipette and tips
P20 pipette and tips
Ice bucket with ice

IMPORTANT

The sequencing reagents outlined in this method are for our most recent V14 chemisty.

If using a previous version of our chemistry or a kit with specific sequencing reagents, please ensure you are using the correct sequencing reagents for your flow cell.

TIP

The buffers used in this process are incompatible with conducting a Flow Cell Check prior to loading a subsequent library. However, the first pore scan once a sequencing run has started will report the number of nanopores available.

IMPORTANT

A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.

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.

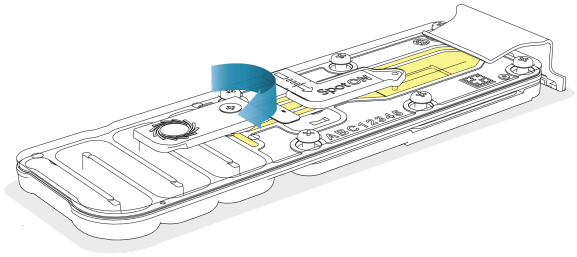
Note: We do not recommend using any other albumin type (e.g. recombinant human serum albumin).

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.**

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

2 Slide open the **Priming port** cover.

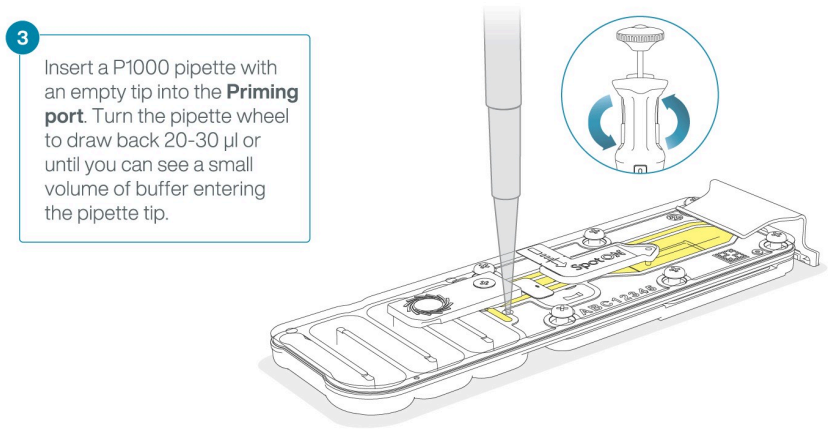


IMPORTANT

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.

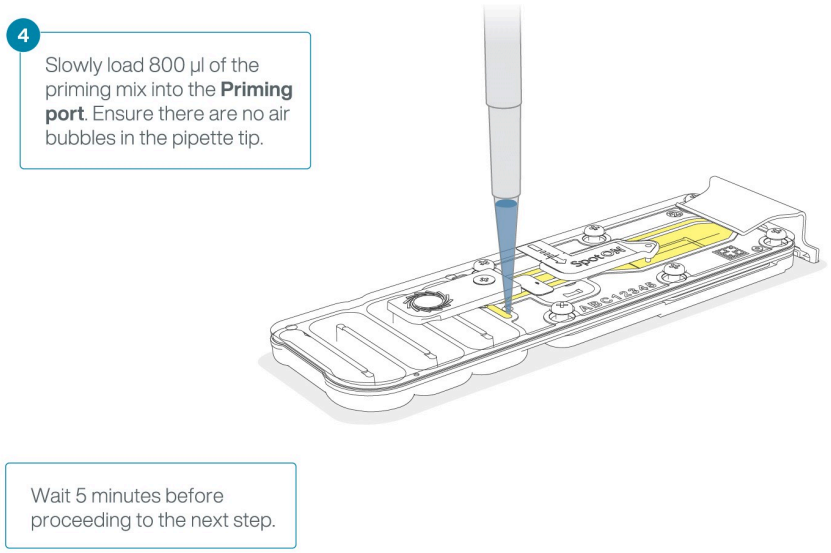
4 After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

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.



5 Slowly load 800 µl of the priming mix into the priming port, as follows:

1. Using a P1000 pipette, take 800 µl of the priming mix
2. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
3. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, as illustrated in the video above, leaving a small volume of buffer in the pipette tip.



IMPORTANT

It is vital to wait five minutes between the priming mix flushes to effectively remove the nuclease.

6 Close the priming port and wait five minutes.

During this time, prepare the library for loading by following the steps below.

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

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.

8 In a new tube, prepare the library for loading according to the "Priming and loading the MinION and GridION Flow Cell" section of the suitable protocol to ensure you are using the correct reagents and volumes.

For Kit 14 chemistry:

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
Recovered DNA library	12 µl
Total	**75 µl**

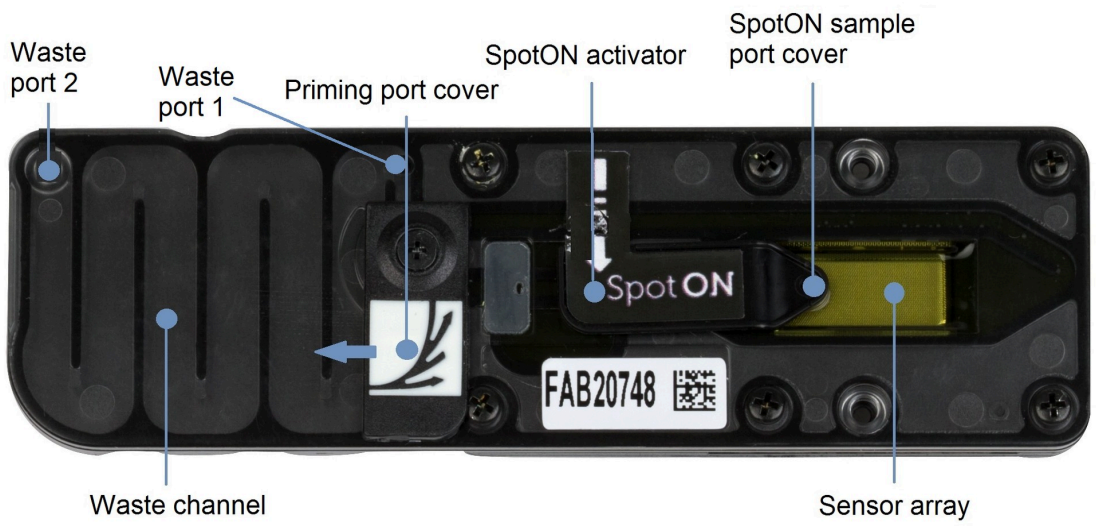
IMPORTANT

It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

9 Remove the waste buffer, as follows:

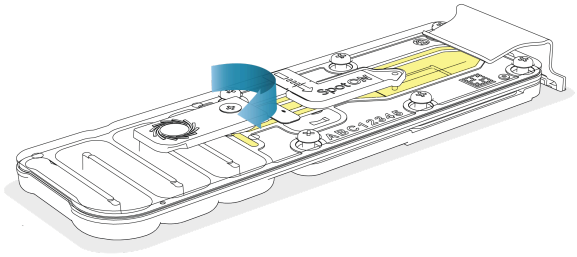
1. Ensure the priming port and SpotON sample port covers are closed, as indicated in the figure below.
2. Insert a P1000 pipette into waste port 1 and remove the waste buffer.

Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



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

2 Slide open the **Priming port** cover.

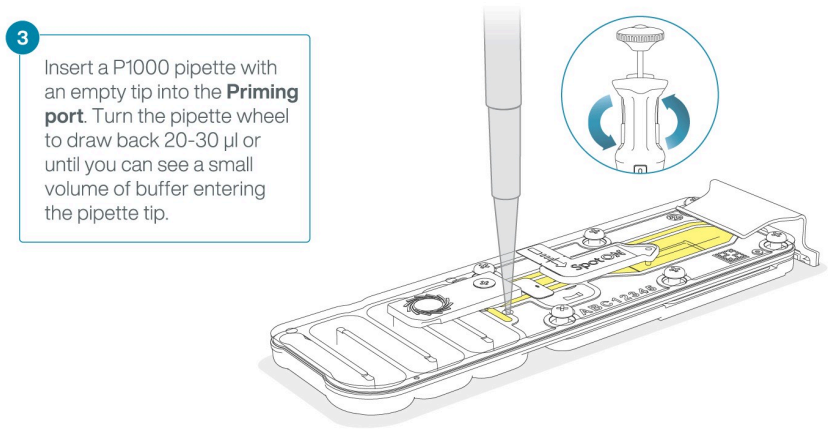


IMPORTANT

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.

11 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.



12 Slowly load 200 µl of the priming mix into the flow cell priming port, as follows:

1. Ensure the priming port is open and gently lift open the SpotON sample port.
2. Using a P1000 pipette, take 200 µl of the priming mix
3. Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip
4. Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger **very slowly**, as illustrated in the video above, leaving a small volume of buffer in the pipette tip.

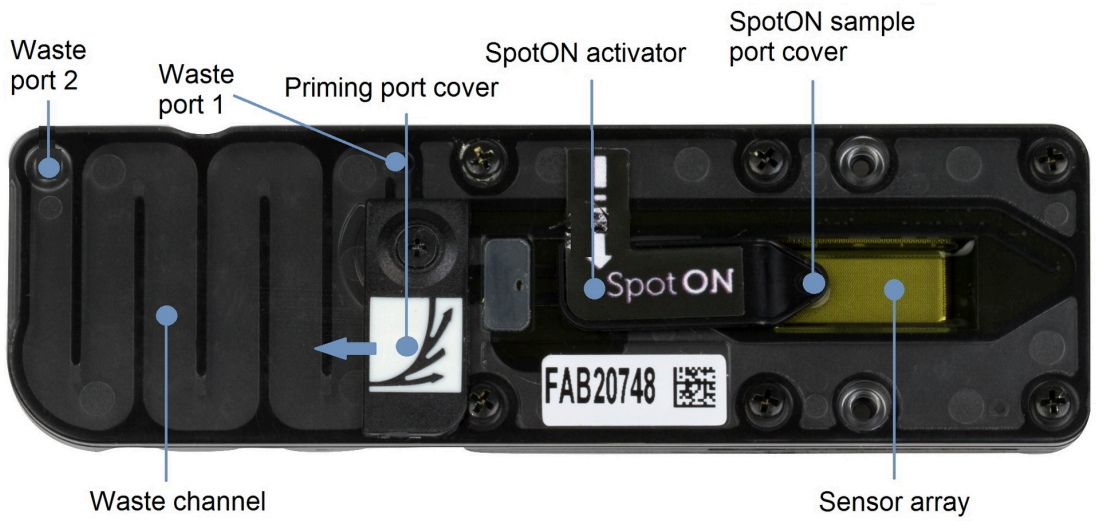
IMPORTANT

It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

13 Remove the waste buffer, as follows:

1. Close the priming port and SpotON sample port cover, as indicated in the figure below.
2. Insert a P1000 pipette into waste port 1 and remove the waste buffer.

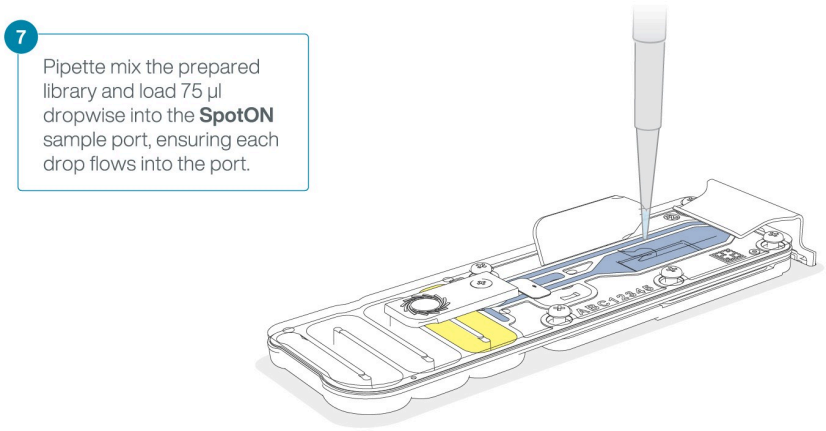
Note: As both the priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



14 Slide open the priming port cover and gently lift open the SpotON sample port cover.

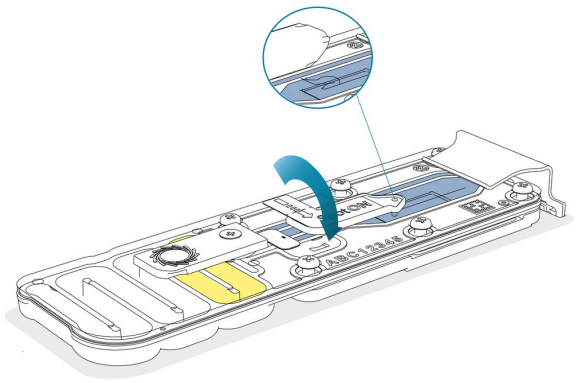
15 Mix the prepared library gently by pipetting up and down just prior to loading.

16 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.

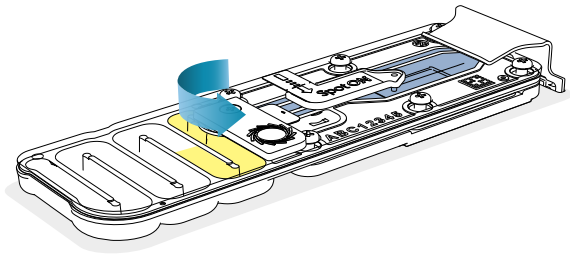


17 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.

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



9 Gently close the **Priming port**.



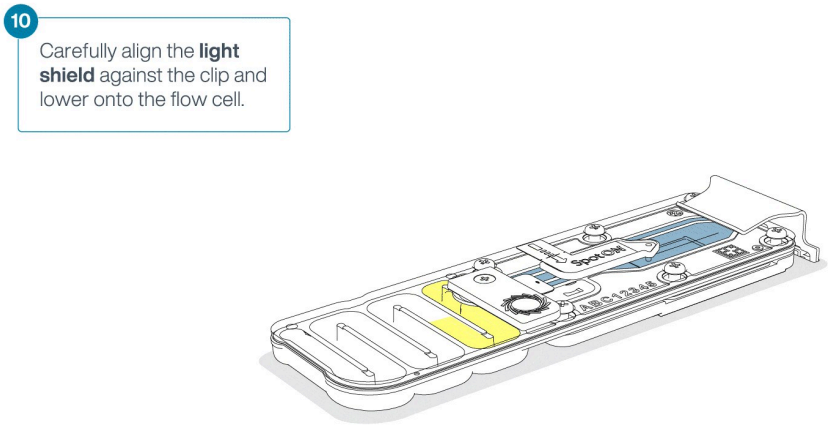
IMPORTANT

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.

18 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 continue sequencing run on MinKNOW.

TIP

Library storage recommendations

We recommend storing libraries in Eppendorf DNA LoBind tubes at **4°C for short term** storage or repeated use, for example, reloading flow cells between washes. For single use and **long-term storage** of more than 3 months, we recommend storing libraries at **-80°C** in Eppendorf DNA LoBind tubes. For further information, please refer to the DNA library stability Know-How document.

5. To store the MinION/GridION Flow Cell for later use

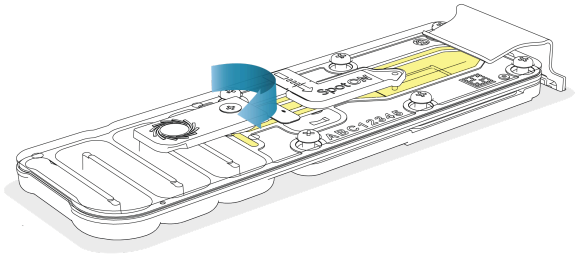
Materials Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)

Equipment P20 pipette and tips
P1000 pipette and tips

Storage Buffer (S) can be used to flush flow cells for storage for later use or to check number of available nanopores before loading another library.

1. **Thaw one tube of Storage Buffer (S) at room temperature.**
2. **Mix contents thoroughly by pipetting and spin down briefly.**
3. **Slide the flow cell priming port cover clockwise to open.**

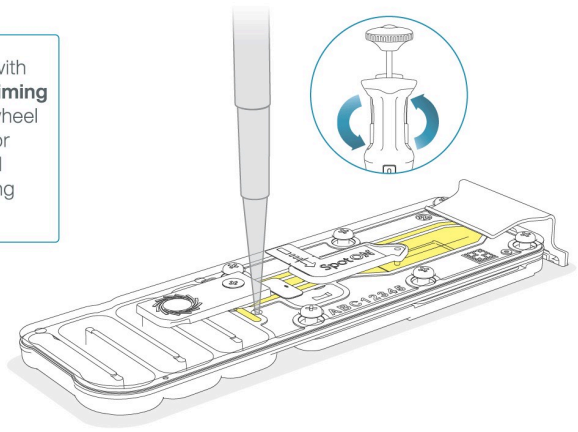
2 Slide open the **Priming port** cover.



4 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 flow cell priming port.
3. Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip.
4. Visually check that there is continuous buffer from the flow cell priming port across the sensor array.

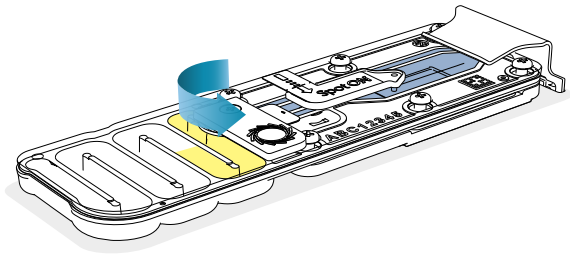
3 Insert a P1000 pipette with an empty tip into the **Priming port**. Turn the pipette wheel to draw back 20-30 µl or until you can see a small volume of buffer entering the pipette tip.



5 **Slowly add 500 µl of Storage Buffer (S) through the flow cell priming port.**

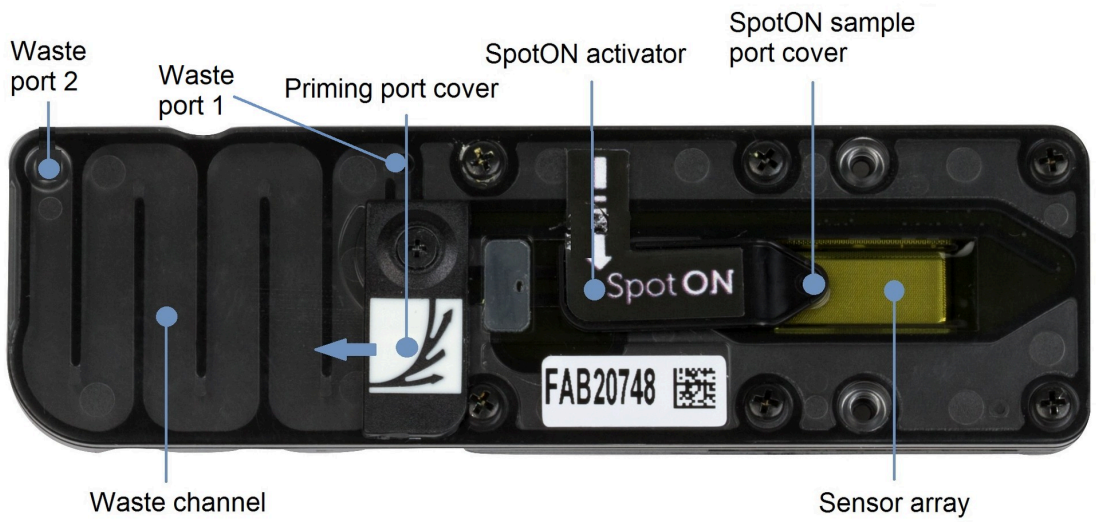
6 **Close the priming port.**

9 Gently close the **Priming port**.



7 **Remove all fluid from the waste channel through waste port 1 using a P1000 pipette.**

As both the flow cell priming port and SpotON sample port are closed, no fluid should leave the sensor array area.



IMPORTANT

It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

8 **The flow cell can now be stored at 4-8°C.**

END OF STEP

When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to room temperature for ~5 minutes.

IMPORTANT

After performing a flow cell wash or storing your flow cell, we recommend using running a 'Flow cell check' to check number of available nanopores.

Load your flow cell into the device with Storage Buffer (S) and start a Flow cell check to detect the number of active pores. For more information, please visit the Flow cell check section of our MinKNOW protocol.

After the Flow cell check, prime your flow cell and load the library before starting a new sequencing run.