

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 washing out the first library, and refreshing the system ready 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 before subsequent library additions. The Flow Cell Wash Kit is compatible with R9.4.1, R10.4 and R10.4.1 flow cells.

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, users may prefer to barcode their libraries when used in conjunction with the Wash Kit, such that reads from different libraries can be separated from each other. RNA is also efficiently removed, and an RNA barcoding option will become available in the near future. Successful deconvolution of DNA reads has been demonstrated in Oxford Nanopore's internal development:

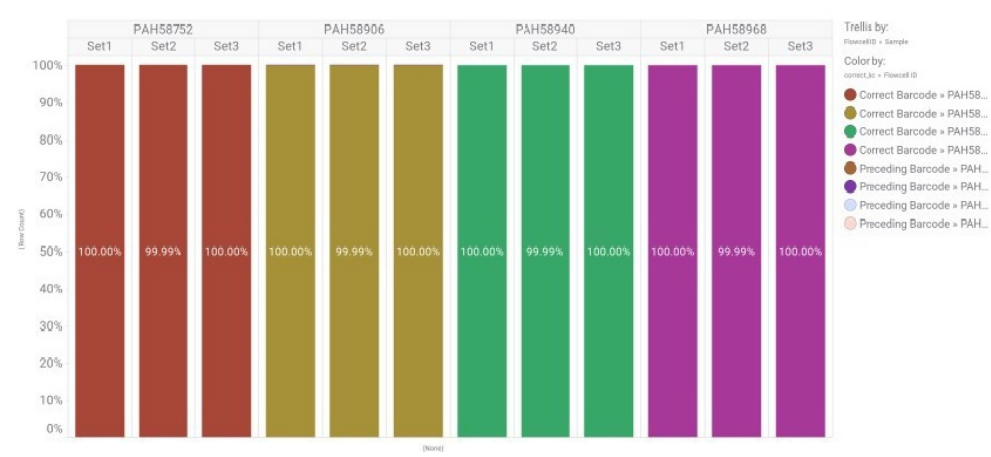


Figure 1. A sample with four barcodes was sequenced before the flow cell was washed using EXP-WSH004 before a sample with four different barcodes was loaded. This was repeated for a third sequencing run. We repeated this using EXP-WSH003 to illustrate the comparable washing between the wash kits.

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:

- 16S Barcoding Kit 1-24 (SQK-16S024)
- 16S Barcoding Kit (SQK-RAB204)
- PCR-cDNA Barcoding Kit (SQK-PCB111.24)
- PCR Barcoding Kit (SQK-PBK004)
- Rapid Barcoding Kit (SQK-RBK004)
- Rapid Barcoding Kit 96 (SQK-RBK110.96)
- Rapid Barcoding Kit 24 V14 (SQK-RBK114.24)
- Rapid Barcoding Kit 96 V14 (SQK-RBK114.96)
- Rapid PCR Barcoding Kit (SQK-RPB004)
- PCR Barcoding Expansion 1-12 (EXP-PBC001)

- PCR Barcoding Expansion 1-96 (EXP-PBC096)
- Native Barcoding Expansion 1-12 (EXP-NBD104)
- Native Barcoding Expansion 13-24 (EXP-NBD114)
- Native Barcoding Expansion 96 (EXP-NBD196)
- Native Barcoding Kit 24 V14 (SQK-NBD114.24)
- Native Barcoding Kit 96 V14 (SQK-NBD114.96)
- Multiplex Ligation Sequencing Kit (SQK-MLK111.96-XL)

Nuclease activity of the Flow Cell Wash Kit

The Flow Cell Wash Kit contains DNase I, which is used to digest any remaining 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 “recovering” state (“unavailable” in the detailed view) (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 asterisks indicate where sequencing has been paused and the flow cell washed. **Please note that 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 only recommended where sequencing channels are lost to the “recovering”/“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](#).

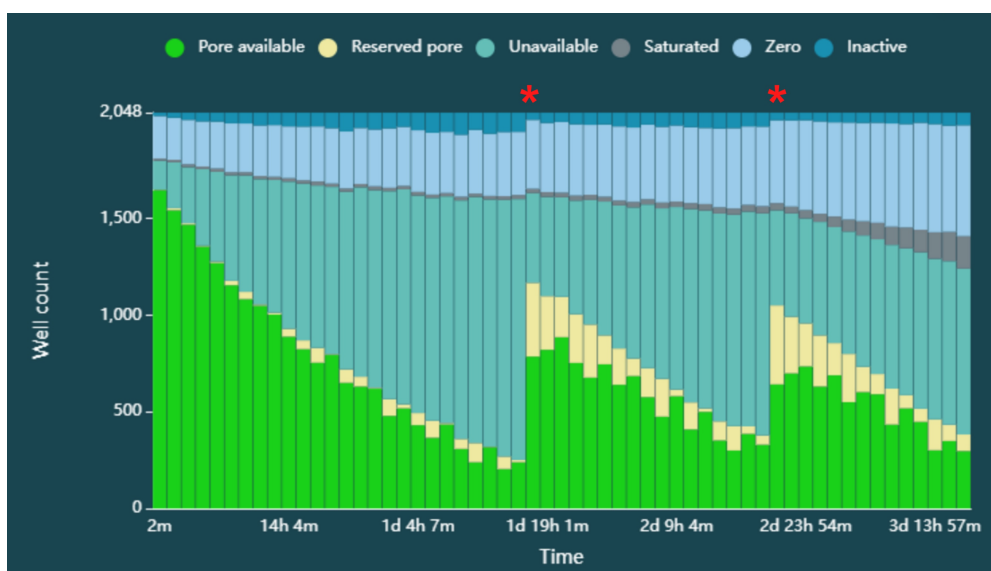


Figure 2. Pore states observed on a flow cell before and after wash steps are performed. A flow cell has been loaded with a sequencing library that has resulted in an accumulation of pores in the “unavailable” state, leading to a decrease in the rate of data acquisition. The red asterisks indicate when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

In experiments where throughput is limited by the increase in pores in the “recovering”/“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 throughput 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 Japanese 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 throughput from the flow cell, without any compromise in observed read length (Figure 4).

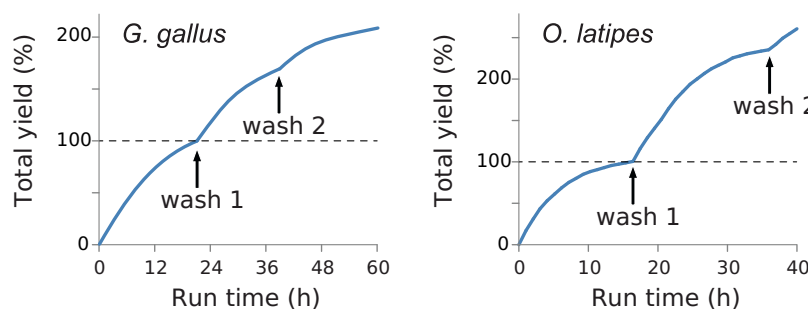


Figure 3. Throughput observed from *Gallus gallus* and *Oryzias latipes* libraries run on a PromethION flow cell and a MinION flow cell, respectively. The arrows indicate the timing of each wash step: wash steps were performed at the point where the rate of data acquisition started to slow due to the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

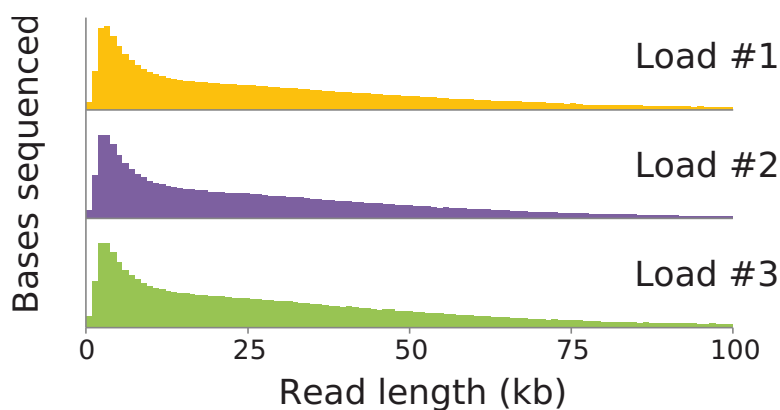


Figure 4. Effective inactivation of the DNase I prevents read length deterioration in the experiments after a nuclease wash is performed. In this example, the read length of the *Gallus gallus* library was recorded before the first wash (load #1) and then again after the first and second washes (load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

Equipment and consumables

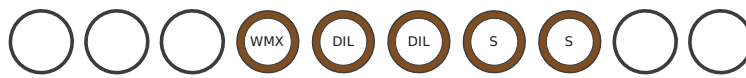
Materials

- Flow Cell Wash Kit (EXP-WSH004)
- Flow cell priming reagents available in your sequencing kit or in the following kits:
- Sequencing Auxiliary Vials V14 (EXP-AUX003) or Sequencing Auxiliary Vials (EXP-AUX002 or EXP-AUX001)
- Flow Cell Priming Kit (EXP-FLP004) or Flow Cell Priming Kit (EXP-FLP002)

Equipment

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

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.

Flushing a PromethION flow cell

Materials

- Flow Cell Wash Kit (EXP-WSH004)

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 and prepare the flow cell for the loading of a subsequent library
- 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) during the wash procedure and also during subsequent library addition
- After the flow cell has been washed, a new library can be loaded or the flow cell can be stored at 4°C

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 clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:

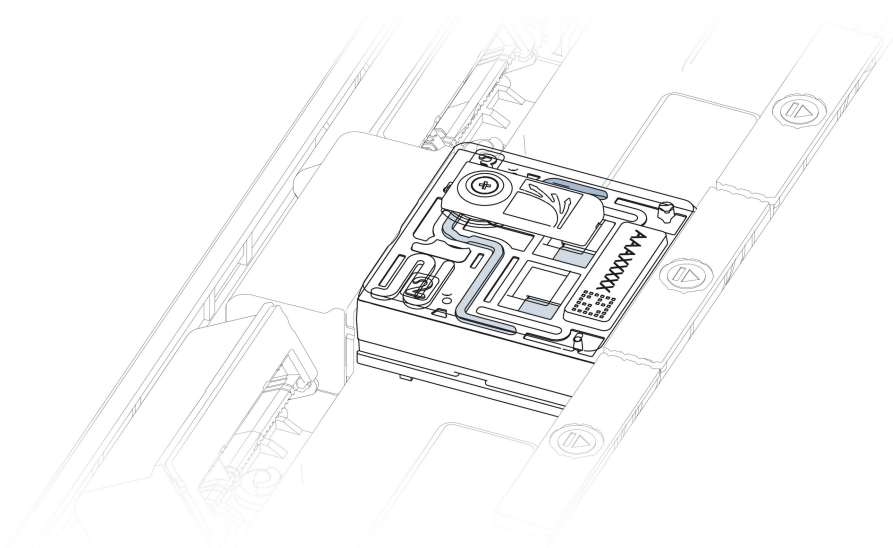
Reagent	Volume per flow cell
Wash Mix (WMX)	2 µl
Wash Diluent (DIL)	398 µl
Total	400 µl

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.

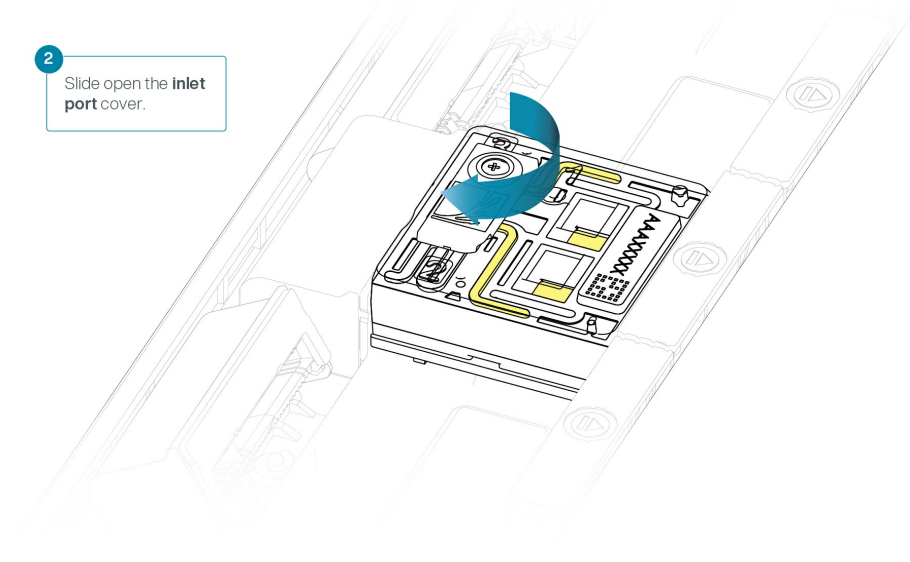
7 Ensure the inlet port is closed and remove any buffer from the waste port.

The waste fluid can be aspirated from either one of the ports, labelled 2 and 3 on the flow cell.



8 Rotate the inlet port cover clockwise to reveal the inlet port.

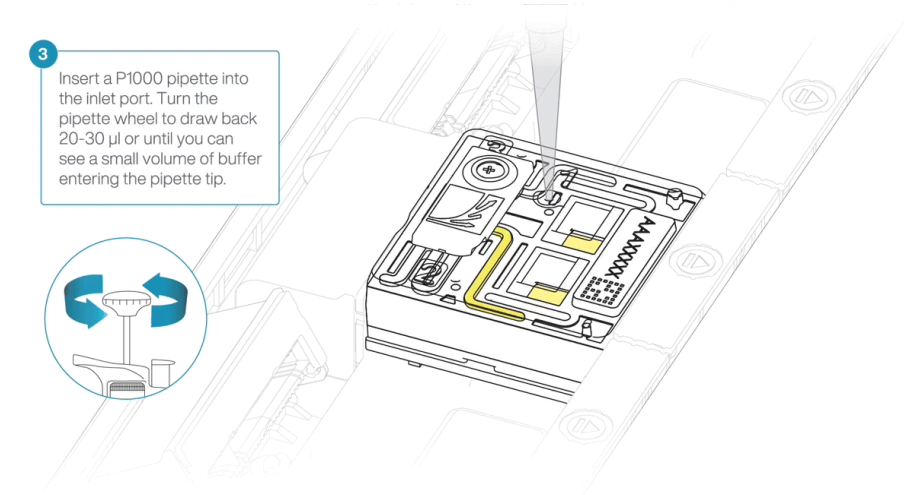
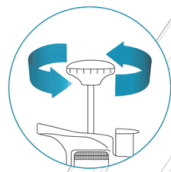
2 Slide open the **inlet port cover**.



9 A small tract of air may be visible beyond the inlet port. If necessary, using a P1000 draw back a small volume to remove any air:

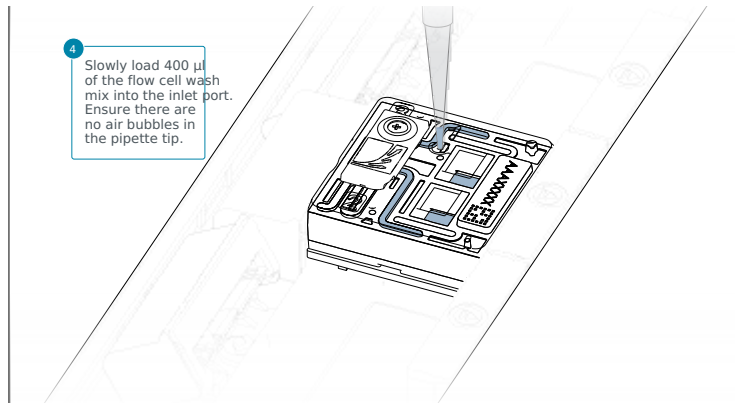
1. Set a P1000 pipette to 200 μl
2. Insert the tip into the inlet port
3. Turn the wheel until the dial shows 220-230 μl , or until you can see a small volume of buffer entering the pipette tip.

3 Insert a P1000 pipette into the inlet 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.

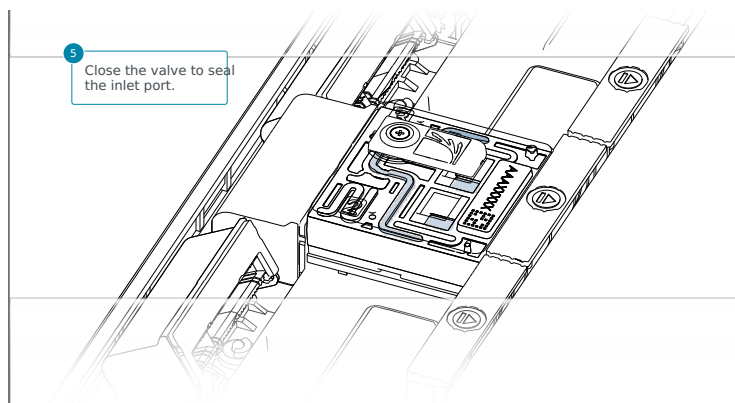


10 Load 400 μ l of the prepared Flow Cell Wash Mix into the flow cell via the inlet port, avoiding the introduction of air.

It may be necessary to use a laboratory wipe to mop up any excess fluid that escapes from the flow cell waste ports 2 and 3.

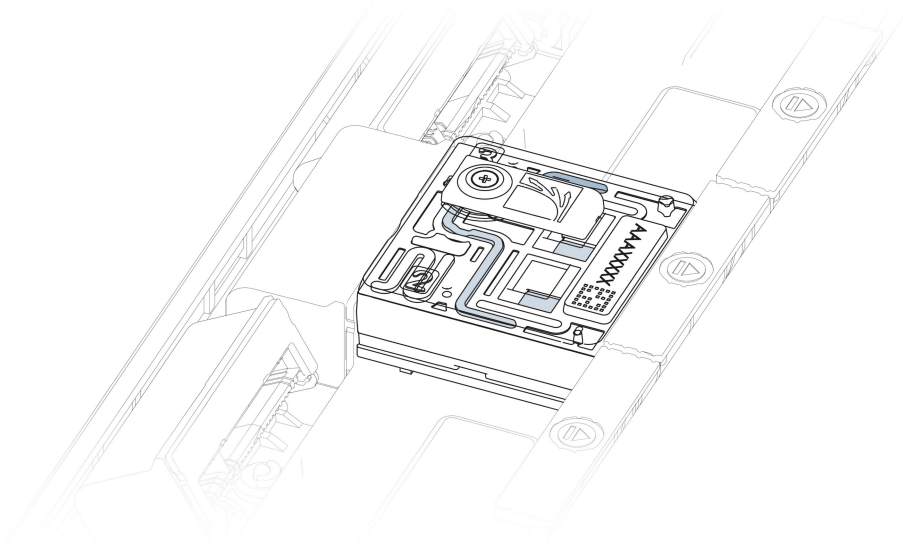


11 Close the inlet port and wait for 60 minutes.



12 Ensure the inlet port is closed and remove buffer from the waste port a second time.

The waste fluid can be aspirated from either one of the ports, labelled 2 and 3 on the flow cell.



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

To run a second library on a PromethION flow cell straight away

Materials

- Flow Cell Wash Kit (EXP-WSH004)
- Flow cell priming reagents available in your sequencing kit or in the following kits:
- Sequencing Auxiliary Vials (EXP-AUX001 or EXP-AUX002 or EXP-AUX003)
- Flow Cell Priming Kit (EXP-FLP004) or Flow Cell Priming Kit (EXP-FLP002)

Equipment

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

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.

1 To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of your library preparation protocol with the recommendations below.

Note: For more consistent and better performance after a flushing a flow cell with the Flow Cell Wash Kit, we recommend the following:

- Pipette very slowly when loading priming mix into the flow cell.
- Wait five minutes between priming mix flushes.
- After the five minute pause, close the inlet port and remove the waste from the waste port. This prevents the nuclease from diffusing through the flow cell. Repeat this step after the second priming mix flush.

As part of this process, the flow cell will need priming using flow cell priming reagents either in your sequencing kit or separately in the Flow Cell Priming Kit.

Once the flow cell has been primed and loaded, either resume the run in MinKNOW or start a new sequencing experiment. **Use an existing** experiment when setting up a sequencing run to use the same settings that were used in a previous experiment.

IMPORTANT

When priming a flow cell after a nuclease wash with the Flow Cell Wash Kit, it is vital to wait five minutes between the priming mix flushes and to remove the waste for effective removal of the nuclease.

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 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 the following kit chemistries:
 - Kit 10 e.g. SQK-LSK110
 - Kit 11 e.g. SQK-PCS111
- [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 V14 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.
- [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.

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](#).

To store the PromethION flow cell for later use

Materials

- Flow Cell Wash Kit (EXP-WSH004)

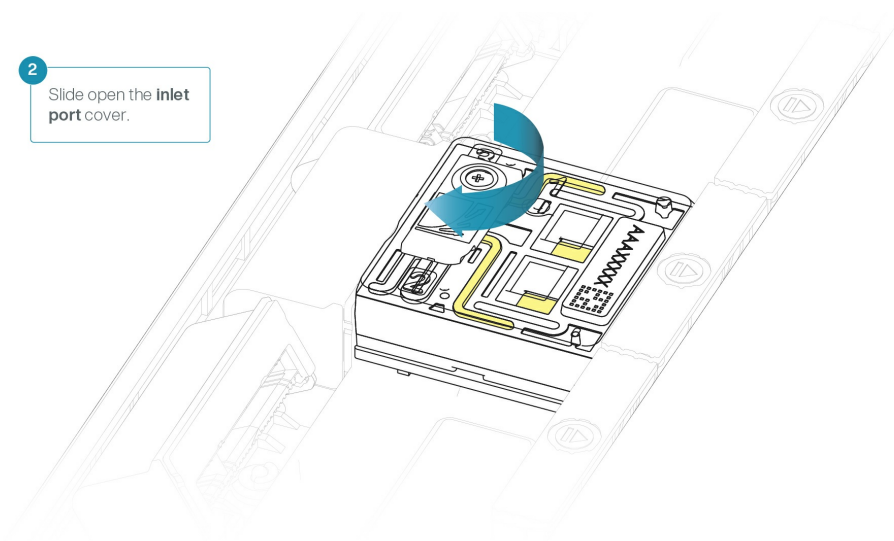
Consumables

- P1000 pipette and tips
- P20 pipette 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.

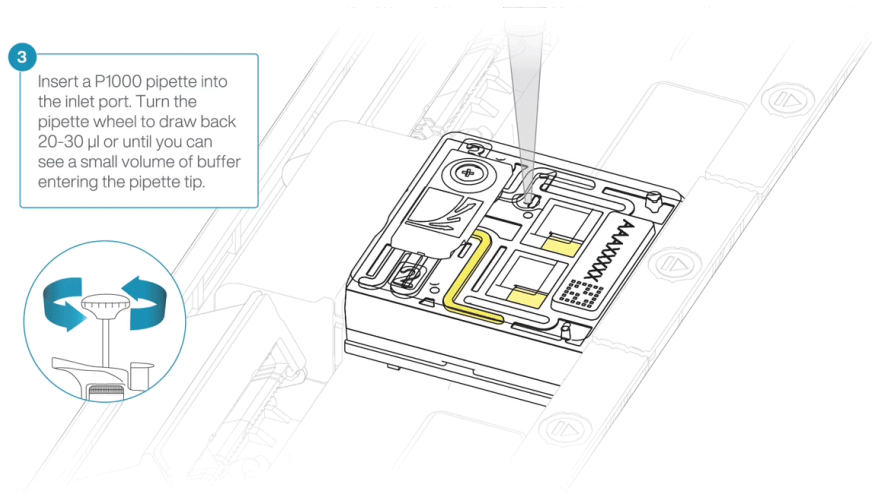
- 1 Thaw one tube of **Storage Buffer (S)** at room temperature.
- 2 Mix contents thoroughly by pipetting and spin down briefly.
- 3 Rotate the inlet port cover clockwise to reveal the inlet port.

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

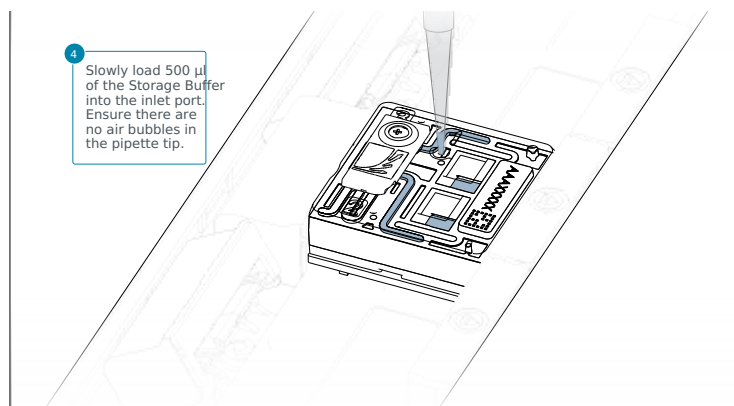


4 A small tract of air may be visible beyond the inlet port. If necessary, using a P1000 draw back a small volume to remove any air:

1. Set a P1000 pipette to 200 μl
2. Insert the tip into the inlet port
3. Turn the wheel until the dial shows 220-230 μl , or until you can see a small volume of buffer entering the pipette tip.

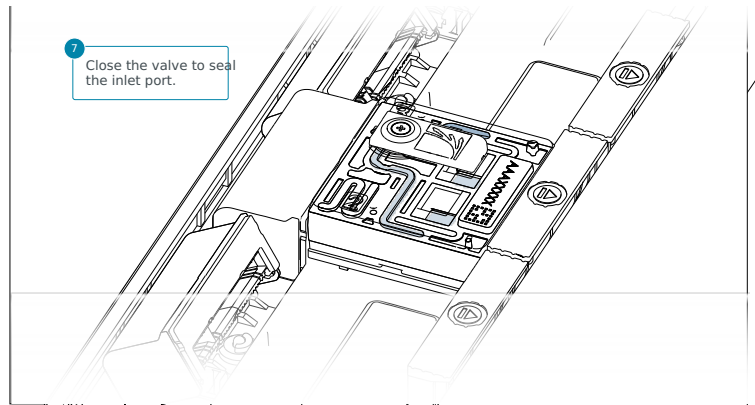


5 Slowly add 500 μl of Storage Buffer through the inlet port of the flow cell.



6 Close the inlet port cover and remove any buffer from the waste port.

The waste liquid can be aspirated from either one of the ports, labelled 2 and 3 on the flow cell.



7 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 the first pore scan to check number of available nanopores.

Load your flow cell into the device with Storage Buffer (S) and start a sequencing run to perform a pore scan to detect the number of active pores. After the pore scan, either:

- stop your sequencing run, prime your flow cell and load the library before starting a new sequencing run.
- or, pause your sequencing run, prime your flow cell and load the library before restarting the sequencing run.