Overview of the protocol

Introduction to the Wash Kit

The 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 and R10.3 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:

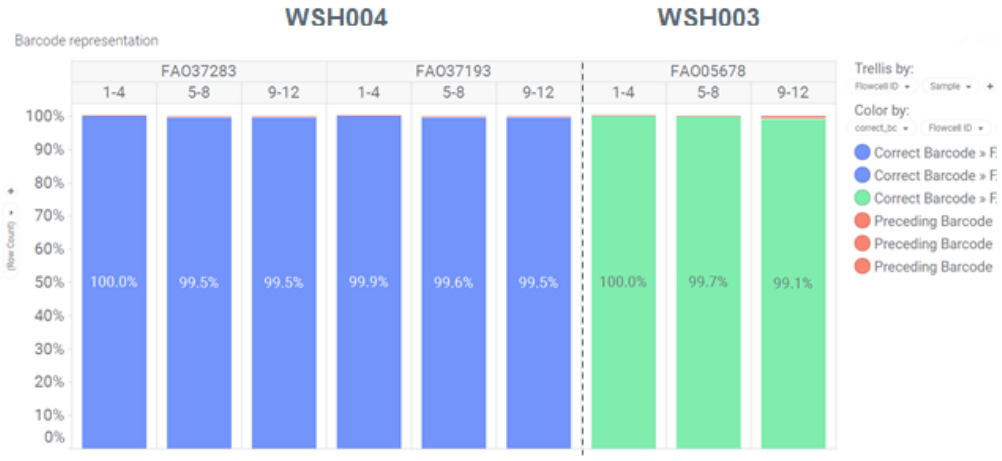


Figure 1. A sample with four barcodes was sequenced before the flow cell was washed using EXP-WSH004 before a sample with four different barcodes was loaded. This was repeated for a third sequencing run. We repeated this using EXP-WSH003 to illustrate the comparable washing between the wash kits.

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
- 16S Barcoding Kit
- PCR-cDNA Barcoding Kit
- PCR Barcoding Kit
- Rapid Barcoding Kit
- Rapid PCR Barcoding Kit
- PCR Barcoding Expansion 1-12
- PCR Barcoding Expansion 1-96
- Native Barcoding Expansion 1-12
- Native Barcoding Expansion 13-24
- Native Barcoding Expansion 96

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 2a) 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 2a, the astrisks 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 Mux scan has been performed.**

The wash step is only recommended where sequencing channels are lost to the “recovering”/”unavailable” state (Figure 2a). In circumstances where channels have been lost by other means, for example “saturated” (Figure 2b), the wash step is not effective at reverting channels to the “active pore” state (Figure 2c).

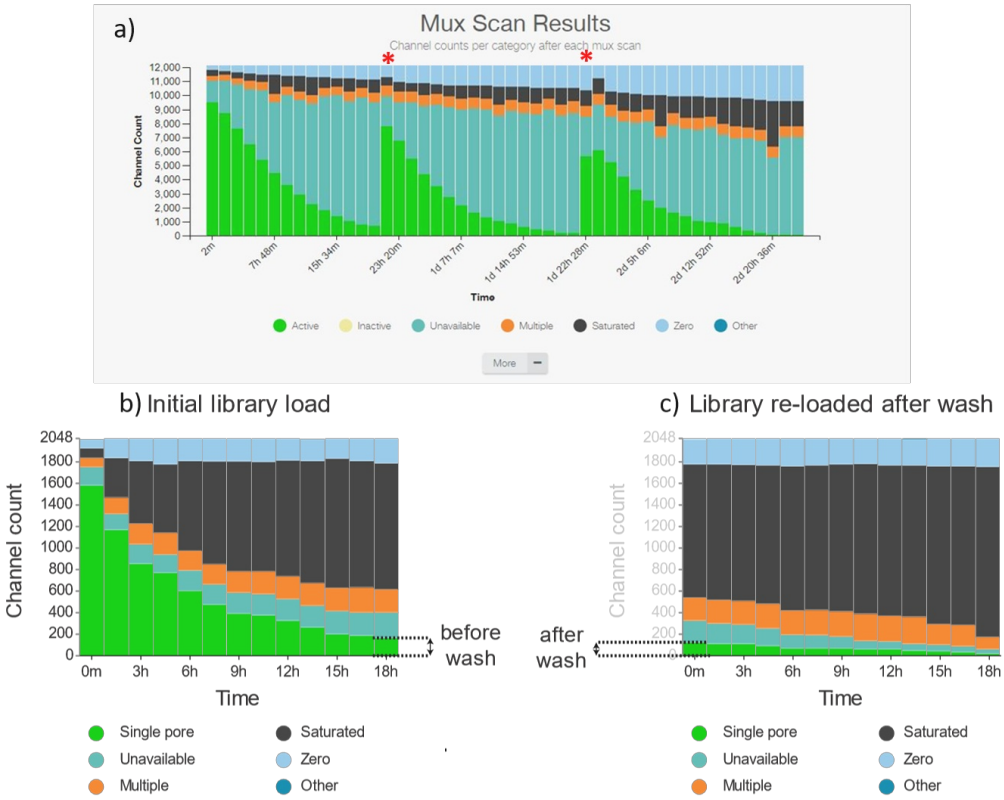


Figure 2. Pore states observed on a PromethION and a MinION flow cell before and after wash steps are performed. **a)** A PromethION flow cell has been loaded with a sequencing library that has resulted in an accumulation of pores in the “unavailable” state, leading to a decrease in the rate of data 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. **b)** A MinION flow cell has been loaded with a sequencing library that has resulted in an accumulation of pores in the “saturated” state, leading to a decrease in the rate of data acquisition. **c)** A wash step has been performed on the MinION flow cell shown in the left panel, but none of the pores that had been lost to the “saturated” state have reverted to the “single pore” state.

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 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 throughput from the flow cell, without any compromise in observed read length (Figure 4).

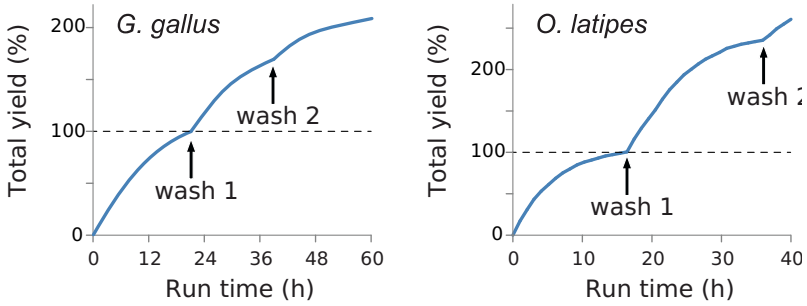


Figure 3. Throughput observed from *Gallus gallus* and *Oryzias latipes* libraries run on a PromethION flow cell and a MinION flow cell, respectively. The arrows indicate the timing of each wash step: wash steps were performed at the point where the rate of data acquisition started to slow due to the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

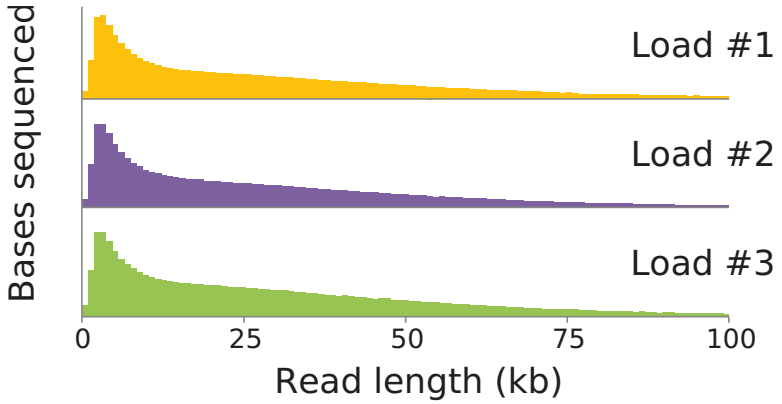


Figure 4. Effective inactivation of the DNase I prevents read length deterioration in the experiments after a nuclease wash is performed. In this example, the read length of the *Gallus gallus* library was recorded before the first wash (load #1) and then again after the first and second washes (load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

Equipment and consumables

Materials	• Flow Cell Wash Kit (EXP-WSH004)
Equipment	• P1000 pipette and tips • P20 pipette and tips • Ice bucket with ice

Flow Cell Wash Kit contents (EXP-WSH004)

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)	1300	2	6
Storage Buffer (S)	1600	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 MinION/GridION 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, 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:**

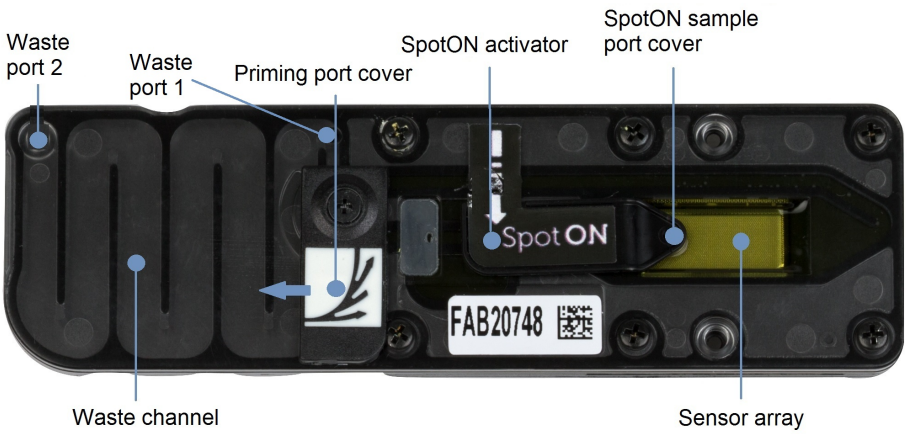
Component	Volume
Wash Mix (WMX)	2 µl
Wash Diluent (DIL)	398 µl
Total	**400 µl**

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 that the priming port cover and SpotON sample port cover are in the positions indicated in the figure below.**

8 **Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the 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 to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

9 **Rotate the flow cell priming port cover clockwise so that the priming port is visible.**

10 Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few µls):

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, 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 priming port across the sensor array.

IMPORTANT

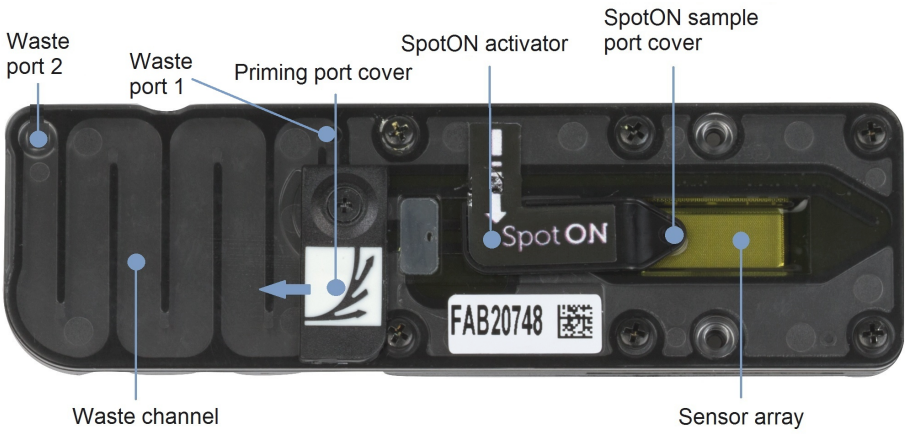
Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, and make sure that the array of pores are covered by buffer at all times. Introducing air bubbles into the array can irreversibly damage pores.

11 Load 400 µl of the prepared Flow Cell Wash Mix into the flow cell via the priming port, avoiding the introduction of air.

12 Close the priming port and wait for 60 minutes.

13 Ensure that the priming port cover and SpotON sample port cover are in the positions indicated in the figure below.

14 Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the 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 to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.

END OF STEP

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

To run a second library on a MinION/GridION flow cell straight away

Materials

- Flow Cell Wash Kit (EXP-WSH004)
- Sequencing Auxiliary Vials (EXP-AUX001)
- Flow Cell Priming Kit (EXP-FLP002)

Equipment

- P1000 pipette and tips
- P20 pipette and tips
- Ice bucket with ice
- MinICN Flow Cell

IMPORTANT

The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading the subsequent library. In order to check your flow cell, follow the instructions in the next section "To store the MinION/GridION/PromethION flow cell for later use" before priming and loading the flow cell.

TIP

For the best results, it is advised to adjust the starting voltage of the new experiment due to voltage drift in the course of the previous run.

The voltage adjustment scheme is described in Adjusting the starting potential for multiple runs in series.

1 **To run a second library straight away, follow the instructions in the "Priming and loading the flow cell" section of the relevant protocol.**

Note: As part of this process the flow cell will need priming using 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.

Reloading a library

Additional buffers for reloading a diluted library, following the washing of a flow cell, 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 prior to '110' chemistry.
- Sequencing Auxiliary Vials expansion (EXP-AUX110). 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 Ligation Sequencing Kit (SQK-LSK110) and other '110' chemistries.

TIP

Library storage recommendations

We recommend storing libraries in Eppendorf LoBind tubes at **4°C for short term** storage or repeated use, for example, re-loading 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 LoBind tubes.
For further information, please refer to the DNA library stability Know-How document.

To store the MinION/GridION flow cell for later use

Materials	• Flow Cell Wash Kit (EXP-WSH004)
Optional Equipment	• P1000 pipette and tips • P20 pipette and tips

1 **Thaw one tube of Storage Buffer (S) at room temperature.**

2 **Mix contents thoroughly by pipetting and spin down briefly.**

3 **Rotate the flow cell priming port cover clockwise so that the priming port is visible.**

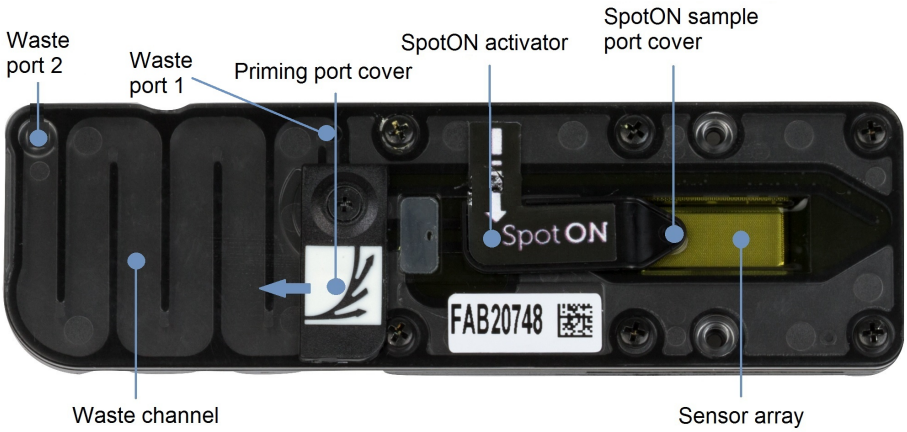
4 **Check for air between the priming port and the sensor array. If necessary, using a P1000 draw back a small volume to remove any air (a few µls):**

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, 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 priming port across the sensor array.

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

6 **Close the priming port.**

7 **Using a P1000, remove all fluid from the waste channel through Waste port 1. As both the 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 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.**

9 **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. You will need to perform a Flow Cell Check before loading the next library.**

TIP

Library storage recommendations

We recommend storing libraries in Eppendorf LoBind tubes at **4°C for short term** storage or repeated use, for example, re-loading flow cells between washes.

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