Washing a MinION flowcell

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1 Works for me

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ABSTRACT

This protocol details the steps required to wash a MInION flowcell using Oxford Nanopore's Flowcell Wash kit (EXP-WSH003). The kit contains a DNase I to digest the previous library and unblock pores to prepare the flowcell membrane for another run.

GUIDELINES

- From experience we have had varying levels of success with flowcell washing (sometimes a flowcell withstands multiple washes, sometime none!), therefore care must be taken to perform each of these steps in order to minimise damage to the membrane.
- Flowcell recovery and subsequent reuse will depend on how long the flowcell was used in the previous run, the type of contamination causing pore blockage and careful application of the wash buffer amongst other factors. It is not guaranteed to work!

MATERIALS

NAME	CATALOG #	VENDOR
Flowcell Wash Kit	EXP-WSH003	Oxford Nanopore Technologies

1 Place the tube of Wash Solution A on ice. **Do not vortex the tube.**

Solution A contains DNase I, which is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube.

2 Thaw one tube of Wash Solution B at room temperature. Mix thoroughly by vortexing, spin down briefly and place on ice.

3 In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Wash Mix:

Component	Volume
Wash Solution A	20 µl
Wash Solution B	380 µl

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

4 If you are still sequencing on the flowcell: stop or pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.

5 **Ensure that the priming port cover and SpotON sample port cover are closed** and remove all fluid from the waste channel using a P1000 pipette.

6 Rotate the flow cell priming port cover clockwise 90° so that the priming port is visible.

7 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:
- Set a P1000 pipette to 200 µl
- Insert the tip into the priming port, holding the pipette perpendicular to the flowcell to ensure no air gets in the port
- Turn the wheel anti-clockwise until the dial shows 220-230 µl, or until you can see a small volume of buffer/liquid entering the pipette tip (do not remove too much liquid or you risk exposing the flowcell to air- a few µl is sufficient)
- Visually check that there is *continuous buffer* from the priming port across the sensor array

8 Load 400 µl of the prepared Wash Mix into the flow cell via the priming port, avoiding the introduction of air. Do this as slowly as possibly- you may want to use the same method of pipette control as above i.e. turning the dial clockwise to dispense the liquid into the flowcell.

9 Close the priming port and wait for 30 minutes.

You can monitor the digestion by starting or resuming a run in MinKNOW. The number of pores in strand should eventually drop to zero and the number of single pores should increase as they unblock.