

# 04\_06\_Washing-of-the-Flow-Cell

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DIENSTAG, 13.7.2021

## Goal-Setting

- Washing of the flow cell so it can be used again with new samples

## Terms / abbreviations

- P1000 = 1000 µL pipette, Eppendorf
- RT = Room temperature

## Risk areas

- None

## Required materials and / or information

- DNA LoBind tube, Eppendorf
- Flow cell wash kit, Nanopore Technologies
  - Wash Mix
  - Wash Diluent
  - Storage Buffer
- Original protocol:

 [Flow Cell Wash Kit \(EXP-WSH004\)-minion.pdf](#)

- Pipettes, Eppendorf

## Templates, devices, software

- Flow cell

## Preliminary work

-  [04\\_04\\_Nanopore-sequencing-with-MinION](#)

## Operation

### Flushing a MinION flow cell

- Place the tube of the Wash Mix on ice. Do not vortex the tube.
- Thaw one tube of Wash Diluent at RT
- Mix the contents of Wash Diluent thoroughly by vortexing, spin down briefly and place on ice
- In a clean 1.5 mL Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:
  - 2 µL Wash Mix
  - 398 µL Wash Diluent
  - Mix well by pipetting, and place on ice. Do not vortex the tube.
- Stop the sequencing experiment in MinKNOW and leave the flow cell in the device. Ensure that the priming port cover and SpotON sample port cover are closed.
- Open the priming port cover by rotating clockwise, so the priming port is visible
- Load 400 µL of the prepared Flow Cell Wash Mix into the flow cell via the priming port, avoiding the introduction of air
- Wait for 10 min

### To run a second library on a MinION flow cell straight away

- Ensure the priming port cover and SpotON sample port cover are closed --> for safety, put a finger on the SpotON sample port cover because air would attack the membrane
- 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
- To run a second library straight away, follow the instructions in the "Priming and loading the SpotON flow cell" section of the SOP: [04\\_04\\_Nanopore-sequencing-with-MinION](#)

### To store the MinION flow cell for later use

- Thaw one tube of Storage Buffer at RT
- Mix contents thoroughly by pipetting and spin down briefly
- Check for air between the priming port and the sensor array after loading the Flow Cell Wash Mix as described above. If necessary, withdraw a small volume with a P1000 to remove air (a few  $\mu\text{L}$ ):
  - Set a P1000 pipette to 200  $\mu\text{L}$
  - Insert the tip into the priming port
  - Turn the wheel until the dial shows 220-230  $\mu\text{L}$ , or until a small volume of buffer/liquid entering the pipette tip can be seen
  - Visually check that there is continuous buffer from the priming port across the sensor array
- Slowly add 500  $\mu\text{L}$  of Storage Buffer through the priming port of the flow cell
- Close the priming port and check if the SpotON sample port is closed --> for safety, put a finger on the SpotON sample port cover because air would attack the membrane
- 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
- The flow cell can now be stored at 4-8 °C
- When reusing the flow cell, remove the flow cell from storage, and allow it to warm to RT for ~5 min. A flow cell check is needed before loading the next library.

## Troubleshooting

- None

## Follow-up work

- None