04_06 Washing of the Flow-Cell

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 µL):
    - Set a P1000 pipette to 200 µL
    - Insert the tip into the priming port
    - Turn the wheel until the dial shows 220-230 µL, 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 µL 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