Loading multiple PromethION Flow Cells

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Flow Cell Number: ..... DNA Samples: .....

Before start checklist

Materials

- [ ] DNA libraries
- [ ] Flow Cell Priming Kit V14 (EXP-FLP004)
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003)
- [ ] Flow Cell Wash Kit XL (EXP-WSH004-XL)

Consumables

- [ ] PromethION Flow Cell (FLO-PRO114M)
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 2 ml Eppendorf DNA LoBind tubes

Equipment

- [ ] PromethION 24/48 device
- [ ] Ice bucket with ice
- [ ] Pipettes and pipette tips P20, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Priming and loading multiple flow cells on a PromethION	
Loading multiple PromethION Flow Cells	
IMPORTANT ☐ Scale up reagent volumes as needed	
IMPORTANT ☐ After taking flow cells out of the fridge, wait 20 minutes before inserting the flow cell into the PromethION for the flow cell to come to RT. Condensation can form on the flow cell in humid environments. Inspect the gold connector pins on the top and underside of the flow cell for condensation and wipe off with a lint-free wipe if any is observed. Ensure the heat pad (black pad) is present on the underside of the flow cell.	
Insert all flow cells into the docking ports within the PromethION: ☐ Line up the first flow cell in position 1A with the connector horizontally and vertically before smoothly inserting into position. ☐ Press down firmly onto the flow cell and ensure the latch engages and clicks into place. ☐ Repeat for the next flow cell until all flow cells are loaded.	
IMPORTANT ☐ Insertion of the flow cells at the wrong angle can cause damage to the pins on the PromethION and affect your sequencing results. If you find the pins on a PromethION position are damaged, please contact support@nanoporetech.com for assistance.	
☐ If not already completed, perform a flow cell check on all flow cells. ☐ Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT, before mixing by vortexing. Then spin down before storing on ice. Prepare the flow cell priming mix in a suitable vial for the number of flow cells to flush. Once combined, mix well by vortexing and keep at RT. ☐ Flow Cell Tether (FCT) 780 µl @ 30 µl (1,500 µl cycle) ☐ Flow Cell Flush (FCF) 30,420 µl @ 1,170 µl (58,500 µl cycles) ☐ Slide the inlet port cover clockwise to open the inlet port of the first flow cell inserted. Repeat for all flow cells.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
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.	
After opening the inlet port, draw back a small volume to remove any air bubbles for all flow cells: ☐ Set a P1000 pipette tip to 200 µl. ☐ Insert the tip into the inlet port of the first flow cell. ☐ Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip. ☐ Repeat for all the flow cells using the same pipette tip. Complete the first flush as follows for all flow cells: ☐ Load 500 µl of the priming mix into the first flow cell via the inlet port, avoiding the introduction of air bubbles. ☐ Start a timer for 5 minutes. This is to ensure 5 minutes passes for the first flow cell before the second flush is started. ☐ Immediately repeat step 1 for the remaining flow cells using the same pipette tip. Complete a second flush once all the flow cells have been flushed once, as follows: ☐ Check that the timer has finished to ensure it has been at least 5 minutes since the first flow cell was flushed. ☐ Load 500 µl of the priming mix into the inlet port of the first flow cell, avoiding the introduction of air bubbles. ☐ Repeat the previous step for the remaining flow cells in the same order as the first flush, using the same pipette tip. ☐ Freshly prepare the DNA libraries for loading as outlined in the relevant protocol. Load your samples: ☐ Gently mix the first library and load the full volume through the inlet port of the first flow cell. ☐ Using a new tip between each flow cell, repeat for the remaining flow cells, ensuring to gently mix each library before loading. ☐ Close the valve to seal the inlet port.	
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	
If the light shield has been removed from the flow cell, install the light shield as follows: ☐ Align the inlet port cut out of the light shield with the inlet port cover on the flow cell. The leading edge of the light shield should sit above the flow cell ID. ☐ Firmly press the light shield around the inlet port cover. The inlet port clip will click into place underneath the inlet port cover.	
Close the PromethION lid when ready to start a sequencing run on MinKNOW.	
☐ For multiple flow cell washing, use the same experiment name and identifying sample IDs for all runs to enable all flow cells to be paused simultaneously.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Washing multiple flow cells on a PromethION	
IMPORTANT ☐ Scale up reagent volumes as needed	
IMPORTANT ☐ A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.	
☐ Place the tube(s) of Wash Mix (WMX) on ice. Do not vortex the tube. ☐ Thaw the tube(s) of Wash Diluent (DIL) at RT. ☐ Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice. Prepare the Wash Mix in a suitable vial for the number of flow cells to wash. Mix well by pipetting, and place on ice. Do not vortex the tube. ☐ Wash Mix (WMX) 52 µl @ 2 µl (100 µl cycles) ☐ Wash Diluent (DIL) 10,348 µl @ 398 µl (19,900 µl cycles) ☐ Stop or pause the sequencing run in MinKNOW for all flow cells to be washed. Leave the flow cells in the device.	
IMPORTANT ☐ It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
Remove the waste buffer, as follows: ☐ Ensure the inlet port is closed. ☐ Insert a P1000 pipette into a waste port and remove the waste buffer ☐ Slide the inlet port cover clockwise to open the inlet port of the first flow cell inserted. Repeat for all flow cells.	
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.	
After opening the inlet port, draw back a small volume to remove any air bubbles: ☐ Set a P1000 pipette to 200 µl. ☐ For the first flow cell, insert the tip into the inlet port. ☐ 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. ☐ Repeat for the remaining flow cells using a new pipette tip. Slowly load 200 µl of the prepared flow cell wash mix into the inlet port, as follows: ☐ Using a P1000 pipette, take 200 µl of the flow cell wash mix ☐ Insert the pipette tip into the inlet port, ensuring there are no bubbles in the tip ☐ Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip. ☐ Set a timer for a 5 minute incubation.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Once the 5 minute incubation time is complete, carefully load the remaining 200 µl of the prepared flow cell wash mix into the inlet port, as follows: ☐ Using a P1000 pipette, take 200 µl of the flow cell wash mix ☐ Insert the pipette tip into the inlet port, ensuring there are no bubbles in the tip ☐ Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip. ☐ Close the inlet port and start a 60 minute timer once the first flow cell has been loaded. ☐ Repeat the two-step addition of the wash mix with the 5 minute break in between and the hour incubation for all of the flow cells. ☐ After each row (8 flow cells), thoroughly mix the Wash Mix.	
IMPORTANT ☐ It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
For each flow cell, remove the waste buffer as follows: ☐ Ensure the inlet port is closed. ☐ Insert a P1000 pipette into a waste port and remove the waste buffer	
Follow one of the two options described in the next steps of the protocol.	
To run a second library on multiple PromethION Flow Cells straight away	
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	
To run a second library straight away, follow the instructions in the 'Priming and loading multiple flow cells on a PromethION' of this protocol, with the recommendations below. ☐ 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 priming port, ensure the SpotON port is closed and remove the waste from waste port 1. This prevents the nuclease from diffusing through the flow cell.	
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.	
To store multiple PromethION Flow Cells for later use	
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.	
IMPORTANT ☐ Scale up reagent volumes as needed	
☐ Thaw Storage Buffer (S) at RT.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Mix contents thoroughly by pipetting and spin down briefly. ☐ Slide the inlet port cover clockwise to open the inlet port of the first flow cell inserted. Repeat for all flow cells.	
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.	
After opening the inlet port, check for a small air bubble under the cover. Draw back a small volume to remove any air bubbles: ☐ Set a P1000 pipette to 200 µl. ☐ For the first flow cell, insert the tip into the inlet port. ☐ Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip. ☐ Repeat for the remaining flow cells. ☐ Slowly add 500 µl of Storage Buffer through the inlet port of the first flow cell and repeat for the remaining flow cells. ☐ Once all the flow cells are loaded, close the inlet port covers, and remove any buffer from the waste port. ☐ Remove the flow cells from the device by pressing down on the latch to release the flow cells before sliding out smoothly. ☐ Store the flow cells at 4-8° C.	
When you wish to reuse the flow cell, remove the flow cell from storage, and allow it to warm to RT for ~5 minutes.	
IMPORTANT ☐ After performing a flow cell wash or storing your flow cell, we recommend using running a 'Flow cell check' to check number of available nanopores.	