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

Before start checklist

Materials

- [ ] Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)
- [ ] Flow cell priming reagents available in your sequencing kit or in the following kits:
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003)
- [ ] Flow Cell Priming Kit (EXP-FLP004)

Consumables

Equipment

- [ ] Ice bucket with ice
- [ ] Pipettes and pipette tips P20, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Flushing a PromethION Flow Cell	
Preparation to run the washing procedure	
IMPORTANT ☐ A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.	
☐ Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube. ☐ Thaw one tube of Wash Diluent (DIL) at RT. ☐ Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice. In a fresh 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix: ☐ 2 µl Wash Mix (WMX) ☐ 398 µl Wash Diluent (DIL) ☐ Mix well by pipetting, and place on ice. Do not vortex the tube. ☐ Stop or pause the sequencing experiment in MinKNOW, and leave the flow cell 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 waste buffer, as follows: ☐ Close the inlet port. ☐ Insert a P1000 pipette into a waste port and remove the waste buffer. ☐ Slide the inlet port cover clockwise to open the inlet port.	
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.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
After opening the inlet port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles: ☐ Set a P1000 pipette to 200 µl ☐ 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. 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. 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 wait for 1 hour.	
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	
Follow one of the two options described in the next steps of the protocol.	
To run a second library on a PromethION Flow Cell straight away	
IMPORTANT ☐ The sequencing reagents outlined in this method are for our most recent V14 chemisty.	
IMPORTANT ☐ A P1000 pipette must be used for all flushing steps to create a seal with the flow cell ports.	
☐ 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 and store on ice. Prepare the flow cell priming mix in a suitable tube for the number of flow cells to flush. Once combined, mix well by briefly vortexing. ☐ 1,170 µl Flow Cell Flush (FCF) ☐ 30 µl Flow Cell Tether (FCT)	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Slide the inlet port cover clockwise to open.	
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 bubbles: ☐ Set a P1000 pipette to 200 µl ☐ 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. Slowly load 500 µl of the priming mix into the inlet port, as follows: ☐ Using a P1000 pipette, take 500 µl of the priming mix ☐ Insert the pipette tip into the priming 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.	
IMPORTANT ☐ It is vital to wait five minutes between the priming mix flushes to ensure effective removal of the nuclease.	
☐ Close the inlet port and wait five minutes. ☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting.	
IMPORTANT ☐ The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	
In a new tube, prepare the library for loading according to the "Priming and loading the PromethION Flow Cell" section of the suitable protocol to ensure you are using the correct reagents and volumes. ☐ 100 µl Sequencing Buffer (SB) ☐ 68 µl Library Beads (LIB) thoroughly mixed before use, or Library Solution (LIS) ☐ 32 µl DNA library	
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.	
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.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
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 load 500 µl of the priming mix into the inlet port, as follows: ☐ Using a P1000 pipette, take 500 µl of the priming mix ☐ Insert the pipette tip into the priming 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.	
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 waste buffer, as follows: ☐ Close the inlet port. ☐ Insert a P1000 pipette into a waste port and remove the waste buffer. ☐ Slide the inlet port cover clockwise to open.	
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 bubbles: ☐ Set a P1000 pipette to 200 µl ☐ 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. ☐ Mix the prepared library gently by pipetting up and down just prior to loading. ☐ Load 200 µl of library into the inlet port using a P1000 pipette. ☐ 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.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Close the PromethION lid when ready to start a sequencing run on MinKNOW.	
To store the PromethION Flow Cell 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. ☐ Thaw one tube of Storage Buffer (S) at RT. ☐ Mix contents thoroughly by pipetting and spin down briefly. ☐ Slide the inlet port cover clockwise to open the inlet port. After opening the inlet port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles: ☐ Set a P1000 pipette to 200 µl ☐ 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. ☐ Slowly add 500 µl of Storage Buffer through the inlet port of the flow cell. ☐ Close the inlet port cover and remove any buffer from the waste port. ☐ The flow cell can now be stored 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.	