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 MinION/GridION 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 clean 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 flow cell priming port and SpotON sample port are closed before removing the waste buffer 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:

- [ ] Close the priming port and SpotON sample port cover, as indicated in the figure below.
- [ ] Insert a P1000 pipette into waste port 1 and remove the waste buffer.

- [ ] Slide the flow cell priming port cover clockwise to open.

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

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 priming 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 flow cell priming 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. ☐ Visually check that there is continuous buffer from the flow cell priming port across the sensor array. Slowly load 200 µl of the prepared flow cell wash mix into the priming port, as follows: ☐ Using a P1000 pipette, take 200 µl of the flow cell wash 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. ☐ Set a timer for a 5 minute incubation. Once the 5 minute incubation is complete, carefully load the remaining 200 µl of the prepared flow cell wash mix into the priming port, as follows: ☐ Using a P1000 pipette, take the remaining 200 µl of the flow cell wash 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. ☐ Close the priming port and wait for 1 hour.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer 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 priming port and SpotON sample port covers are closed, as indicated in the figure below. ☐ Insert a P1000 pipette into waste port 1 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 MinION/GridION 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.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ 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.	
IMPORTANT ☐ For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.	
To prepare the flow cell priming mix with BSA, combine Flow Cell Flush (FCF) and Flow Cell Tether (FCT), as directed below. Mix by pipetting at RT. ☐ 1,170 µl Flow Cell Flush (FCF) ☐ 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml ☐ 30 µl Flow Cell Tether (FCT) ☐ Slide the flow cell priming port cover clockwise to open the priming 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.	
After opening the priming 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 priming port ☐ Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip Note: Visually check that there is continuous buffer from the priming port across the sensor array. Slowly load 800 µl of the priming mix into the priming port, as follows: ☐ Using a P1000 pipette, take 800 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, as illustrated in the video above, leaving a small volume of buffer in the pipette tip.	
IMPORTANT ☐ It is vital to wait five minutes between the priming mix flushes to effectively remove the nuclease.	
☐ Close the priming 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 MinION and GridION Flow Cell" section of the suitable protocol to ensure you are using the correct reagents and volumes. ☐ 37.5 µl Sequencing Buffer (SB) ☐ 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using ☐ 12 µl Recovered DNA library	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer 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 priming port and SpotON sample port covers are closed, as indicated in the figure below. ☐ Insert a P1000 pipette into waste port 1 and remove the waste buffer. ☐ Slide the flow cell priming 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 priming 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 priming port ☐ Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip Note: Visually check that there is continuous buffer from the priming port across the sensor array. Slowly load 200 µl of the priming mix into the flow cell priming port, as follows: ☐ Ensure the priming port is open and gently lift open the SpotON sample port. ☐ Using a P1000 pipette, take 200 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, as illustrated in the video above, leaving a small volume of buffer in the pipette tip.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer 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: ☐ Close the priming port and SpotON sample port cover, as indicated in the figure below. ☐ Insert a P1000 pipette into waste port 1 and remove the waste buffer. ☐ Slide open the priming port cover and gently lift open the SpotON sample port cover. ☐ Mix the prepared library gently by pipetting up and down just prior to loading. ☐ Add 75 µl of the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next. ☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	
Place the light shield onto the flow cell, as follows: ☐ Carefully place the leading edge of the light shield against the clip. Note: Do not force the light shield underneath the clip. ☐ Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.	
Close the device lid and continue sequencing run on MinKNOW.	
To store the MinION/GridION 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 flow cell priming port cover clockwise to open. After opening the priming 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 flow cell priming 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. ☐ Visually check that there is continuous buffer from the flow cell priming port across the sensor array. ☐ Slowly add 500 µl of Storage Buffer (S) through the flow cell priming port. ☐ Close the priming port. ☐ Remove all fluid from the waste channel through waste port 1 using a P1000 pipette.	
IMPORTANT ☐ It is vital that the flow cell priming port and SpotON sample port are closed before removing the waste buffer to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
☐ 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.	