Library recovery from flow cells

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

Before start checklist

Materials

- [ ] Flow Cell Priming Kit (EXP-FLP004)
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003)
- [ ] Flow Cell Wash Kit (EXP-WSH004) or Flow Cell Wash Kit XL (EXP-WSH004-XL)
- [ ] SFB Expansion (EXP-SFB001)

Consumables

- [ ] MinION Flow Cell (FLO-MIN106 or FLO-MIN114)
- [ ] Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] Agencourt AMPure XP beads (Beckman Coulter™ cat # A63881)
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)

Equipment

- [ ] MinION or GridION device
- [ ] P200 pipette and tips
- [ ] Ice bucket with ice
- [ ] Vortex mixer
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Microfuge
- [ ] Magnetic rack
- [ ] Heating block
- [ ] Qubit fluorometer (or equivalent)
- [ ] Pipettes and pipette tips P20, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS

Transfer a library between MinION flow cells

Preparation to transfer a library to a second flow cell

IMPORTANT

- [ ] We recommend keeping the light shield on the original flow cell when recovering the library.

- [ ] Stop the sequencing run for the original flow cell on MinKNOW by clicking 'Stop'.
- [ ] Thaw and prepare the flow cell priming mix according to the "Priming and loading the SpotON flow cell" section of the suitable protocol.
- [ ] Open the MinION or GridION device lid and slide the second flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.
- [ ] To prime the second flow cell, slide the 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.

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

INSTRUCTIONS	NOTES/OBSERVATIONS
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. ☐ Load 800 µl of the priming mix into the second flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. Complete the flow cell priming for the second flow cell: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles. ☐ To prepare the original flow cell for library recovery, slide open the priming port cover and lift open the SpotON sample port cover. ☐ Set a pipette to 75 µl and fully depress the pipette before inserting the tip into the SpotON port of the original flow cell. Slowly aspirate to recover the DNA library from the flow cell. ☐ Add the recovered DNA library to the second 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 of the second flow cell, making sure the bung enters the SpotON port and close the priming port.	
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. ☐ The original flow cell can be flushed with deionised water and returned to Oxford Nanopore. ☐ Start a new sequencing run on MinKNOW for the second flow cell.	
Using the suitable protocol for your DNA library, continue with the "Sequencing and data analysis" section to complete the experiment.	
Clean up and transfer a library between MinION Flow Cells	
Preparation to clean up a library before transfer to a second flow cell	
IMPORTANT ☐ We recommend keeping the light shield on the original flow cell when recovering the library.	

Flow Cell Number: ....

DNA Samples: ....

INSTRUCTIONS	NOTES/OBSERVATIONS

Thaw the kit components at RT and prepare as indicated by the table below:

- [ ] AMPure XP Beads (AXP)
  - 1. Thaw at RT: ⍰
  - 2. Mix well by vortexing: ⍰
  - 3. Briefly spin down: X
  - 4. Keep on ice: X Keep at RT
- [ ] Short Fragment Buffer (SFB)
  - 1. Thaw at RT: ⍰
  - 2. Mix well by vortexing: ⍰
  - 3. Briefly spin down: ⍰
  - 4. Keep on ice: ⍰
- [ ] Elution Buffer (EB)
  - 1. Thaw at RT: ⍰
  - 2. Mix well by vortexing: ⍰
  - 3. Briefly spin down: ⍰
  - 4. Keep on ice: ⍰

- [ ] Stop the sequencing run for the original flow cell on MinKNOW by clicking 'Stop'.
- [ ] To prepare the original flow cell for library recovery, slide open the priming port cover and lift open the SpotON sample port cover.
- [ ] Set a pipette to 75 µl and fully depress the pipette before inserting the tip into the SpotON port of the original flow cell. Slowly aspirate to recover the DNA library from the flow cell.
- [ ] Transfer the recovered library to a fresh 1.5 ml Eppendorf DNA LoBind tube and store on ice.
- [ ] The original flow cell can be removed from the MinION or GridION device by sliding the flow cell from under the clip.
- [ ] The original flow cell can be flushed with deionised water and returned to Oxford Nanopore.
- [ ] Resuspend the AMPure XP Beads (AXP) by vortexing.
- [ ] Add 300 µl of resuspended AMPure XP Beads (AXP) to the recovered library and mix by flicking.
- [ ] Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT.
- [ ] Spin down the sample and pellet on a magnet until supernatant is clear and colourless. Keep the tube on the magnet, and pipette off the supernatant.
- [ ] Wash the beads by adding 150 µl of Short Fragment Buffer (SFB). Flick the beads to resuspend, spin down, then return the tube to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard.
- [ ] Spin down and place the tube back on the magnet. Pipette off any residual supernatant.
- [ ] Remove the tube from the magnetic rack and resuspend the pellet in 13 µl of Elution Buffer (EB).
- [ ] Spin down and incubate for 10 minutes at RT. For high molecular weight DNA, incubating at 37°C can improve recovery of long fragments.
- [ ] Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.
- [ ] Remove and retain 13 µl of eluate containing the DNA library into a clean 1.5 ml Eppendorf DNA LoBind tube.

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

INSTRUCTIONS	NOTES/OBSERVATIONS
Quantify 1 µl of eluted sample using a Qubit fluorometer. If the recovered library is below the detection level of the Qubit dsDNA HS Assay, we do not recommend continuing to load the flow cell.	
☐ The library can be stored at 4°C. ☐ Thaw and prepare the flow cell priming mix according to the "Priming and loading the SpotON flow cell" section of the suitable protocol. ☐ Open the MinION or GridION device lid and slide the second flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact. ☐ To prime the second flow cell, slide the 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. ☐ Load 800 µl of the priming mix into the second flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. ☐ Thoroughly mix the contents of the Library Beads/Loading Beads by pipetting.	
IMPORTANT ☐ The Library Beads/Loading Beads 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 recovered library for loading according to the "Priming and loading the SpotON 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 Complete the flow cell priming for the second flow cell: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles. ☐ Mix the prepared library gently by pipetting up and down just prior to loading. ☐ Add the recovered DNA library to the second flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Gently replace the SpotON sample port cover of the second flow cell, making sure the bung enters the SpotON port and close the priming port.	
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. ☐ Start a new sequencing run on MinKNOW for the second flow cell.	
Using the suitable protocol for your DNA library, continue with the "Sequencing and data analysis" section to complete the experiment.	
Recover a library to replace on a washed MinION flow cell	
Preparation to recover and wash a library to replace on the same flow cell	
IMPORTANT ☐ We recommend keeping the light shield on the flow cell during library recovery, washing and reloading for optimal sequencing output.	
☐ Pause the sequencing run for the original flow cell on MinKNOW by clicking 'Pause'. ☐ Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube. ☐ Thaw one tube of Wash Diluent (DIL) at RT and mix the contents of Wash Diluent (DIL) thoroughly by vortexing. Then spin down briefly and place on ice. ☐ To prepare the original flow cell for library recovery, slide open the priming port cover.	
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.	
IMPORTANT ☐ Be aware that the library is removed from the priming port as a larger volume and expect to see the fluid in the waste channel to move back.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Set a pipette to 150 µl and fully depress the pipette before inserting the tip into the priming port of the original flow cell. Slowly aspirate to recover the DNA library from the flow cell. ☐ Transfer the recovered library to a fresh 1.5 ml Eppendorf DNA LoBind tube and store 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.	
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. ☐ Rotate the flow cell priming port cover clockwise so that the priming port is visible.	
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.	

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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: ☐ 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. ☐ Thaw and prepare the flow cell priming mix according to the "Priming and loading the SpotON flow cell" section of the suitable protocol. ☐ 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. ☐ Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. Complete the flow cell priming: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles. ☐ Bring the recovered library up to RT and load 150 µl to the flow cell via the SpotON 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.	
IMPORTANT ☐ If the light shield was removed during the washing step, the light shield should be replaced on the flow cell as soon as library is 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.	

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

INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Restart the sequencing run on MinKNOW.	
Using the suitable protocol for your DNA library, continue with the "Sequencing and data analysis" section to complete the experiment.	