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

## Before start checklist

### Materials

- [ ] 10 µg of high molecular weight genomic DNA or 5 x $10^6$ cells (e.g. cell culture or tissue)
- [ ] Short Fragment Eliminator (EXP-SFE001) kit
- [ ] Ligation Sequencing Kit V14 (SQK-LSK114)
- [ ] Flow Cell Wash Kit (EXP-WSH004)
- [ ] Sequencing Auxiliary Vials V14 (EXP-AUX003)

### Consumables

- [ ] NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (cat # E7180S or E7180L). Alternatively, you can use the NEBNext® products below:
- [ ] NEBNext FFPE Repair Mix (NEB, M6630)
- [ ] NEBNext® Ultra™ II End Repair/dA-Tailing Module (E7546)
- [ ] NEBNext Quick Ligation Module (NEB, E6056)
- [ ] Puregene Cell Kit (QIAGEN, Cat. # 158043)
- [ ] Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- [ ] 15 ml Falcon tubes
- [ ] 2 ml Eppendorf DNA LoBind tubes
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] Freshly prepared 70% ethanol in nuclease-free water
- [ ] Phosphate Buffered Saline (PBS), pH 7.4 (Thermo Fisher, 10010023)
- [ ] Isopropanol, 100% (Fisher, 10723124)
- [ ] TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Fisher scientific, 10224683)
- [ ] Inoculation loop or disposable tweezers
- [ ] (Optional) TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Qubit dsDNA BR Assay Kit (Invitrogen, Q32850)
- [ ] Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)
- [ ] Agilent Genomic DNA 165 kb Analysis Kit

### Equipment

- [ ] Hula mixer (gentle rotator mixer)
- [ ] Magnetic rack, suitable for 1.5 ml Eppendorf tubes
- [ ] Microfuge
- [ ] Vortex mixer
- [ ] Thermal cycler
- [ ] Centrifuge and rotor suitable for 15 ml Falcon tubes
- [ ] Incubator or water bath set at 37°C and 50°C
- [ ] P2 pipette and tips
- [ ] Ice bucket with ice
- [ ] Timer
- [ ] Heating block
- [ ] Ice bucket with ice
- [ ] Agilent Femto Pulse System (or equivalent for read length QC)
- [ ] Qubit fluorometer (or equivalent for QC check)
- [ ] Pipettes and pipette tips P10, P20, P100, P200, P1000

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| **Human cell line DNA extraction using the QIAGEN Puregene Cell Kit** | |
| IMPORTANT<br>☐ We recommend using wide-bore pipette tips during sample and library preparation for N50s >20 kb to avoid mechanical shearing of your sample. | |
| ☐ Prepare a 1.5 ml Eppendorf DNA LoBind tube with approximately 1 ml of 70% ethanol and store on ice to cool. | |
| ☐ We recommend to harvest and pellet 5 x $10^6$ cells in a 1.5 ml Eppendorf DNA LoBind tube. However, this may vary depending on your sample type. If any liquid remains associated with the pellet, spin down the cells again, then aspirate and discard the remaining supernatant. | |
| ☐ Add 200 µl of 1x PBS to pelleted cells. | |
| ☐ Centrifuge at 300 x g for 3 minutes. | |
| ☐ Without disturbing the pellet, aspirate and discard the supernatant. | |
| ☐ Add 2 ml of Cell Lysis Solution to the washed cell pellet and resuspend the cells using a wide-bore pipette tip. | |
| ☐ Transfer the resuspended cells to a 15 ml Falcon. If clumps of cells remain, gently invert the tube to ensure resuspension. | |
| ☐ Incubate the sample at 37°C for 30 minutes. | |
| ☐ Add 700 µl of the Protein Precipitation Solution to the lysed cells and mix by vortexing for three pulses of 5 seconds. | |
| ☐ Centrifuge the sample at 2000 x g for 5 minutes. | |
| ☐ Transfer the supernatant to a new 15 ml falcon tube and add 2.5 ml isopropanol at RT. Discard the pellet. | |
| ☐ Mix by gently inverting the tube 50 times. | |
| ☐ Spool the DNA using an inoculation loop or disposable tweezers. | |
| ☐ Briefly dip the spooled DNA in the 1.5 ml Eppendorf DNA LoBind tube containing cold 70% ethanol and allow to air dry for a few seconds. | |
| ☐ Transfer the inoculation loop or tweezers with the spooled DNA to a 1.5 ml Eppendorf DNA LoBind tube containing 250 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and allow the DNA to gently dislodge from the loop/tweezers. | |
| ☐ Incubate the DNA pellet for 2 hours at 50°C, occasionally mixing the tube by gentle inversion to aid dissolving the pellet. Alternatively, the DNA pellet can be left overnight at RT. | |
| ☐ Quantify the sample three times using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to the next step. | |
| Take the sample in 250 µl TE buffer forwards into the next step or the sample can be stored at 4°C overnight. | |

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

| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| **Size select for 30 kb input using Short Fragment Eliminator kit** | |
| ☐ In a 1.5 ml Eppendorf DNA LoBind tube, prepare 10 µg DNA in 100 µl of Nuclease-free water to a final concentration of ~100 ng/µl.<br>☐ Add an equal volume of the Short Fragment Eliminator (SFE) buffer to the DNA sample and mix thoroughly by gently flicking the tube until homogenous.<br>☐ Place the tube in the centrifuge and note the orientation of the tube within the rotor.<br>☐ Centrifuge the sample at 10,000 x g at RT for 30 minutes.<br>☐ Aspirate and discard the supernatant, taking care not to disturb the pellet.<br>☐ Without disturbing the pellet, add 200 µl of freshly prepared 70% ethanol to the tube. Centrifuge the sample at 10,000 x g for 3 minutes at the same orientation used in for the previous centrifuge step. Pipette off the ethanol and discard.<br>☐ Repeat the previous step.<br>☐ Add 50 µl of Nuclease-free water to the DNA pellet and mix by gently flicking the tube.<br>☐ Incubate the tube in a heat block at 37°C for 30 minutes. Gently agitate the solution by flicking every 5 minutes to aid with resuspension. Alternatively, use an incubated shaking heat block at 37°C, 300 rpm for 30 minutes.<br>☐ Gently mix the tube contents by pipetting up and down using a wide-bore tip.<br>☐ Quantify the sample three times using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to library preparation. | |
| Quantify sample read length using the Agilent Femto Pulse System. An N50 of 30 kb or above is to be expected. | |
| Take forwards 3 µg of sample into the library preparation step or the sample can be stored at 4°C overnight. | |
| **DNA repair and end-prep** | |
| IMPORTANT<br>☐ We recommend using wide-bore pipette tips during sample and library preparation for N50s >20 kb to avoid mechanical shearing of your sample. | |
| ☐ Thaw DNA Control Sample (DCS) at RT, spin down, mix by pipetting, and place on ice. | |

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
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Prepare the NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice.

- ☐ Thaw all reagents on ice.
- ☐ Flick and/or invert the reagent tubes to ensure they are well mixed.
  Note: Do not vortex the FFPE DNA Repair Mix or Ultra II End Prep Enzyme Mix.
- ☐ Always spin down tubes before opening for the first time each day.
- ☐ The Ultra II End Prep Buffer and FFPE DNA Repair Buffer may have a little precipitate. Allow the mixture to come to RT and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for 30 seconds to solubilise any precipitate.
  Note: It is important the buffers are mixed well by vortexing.
- ☐ The FFPE DNA Repair Buffer may have a yellow tinge and is fine to use if yellow.

Prepare the extracted gDNA in Nuclease-free water:

- ☐ Transfer 3 µg of gDNA into a 1.5 ml Eppendorf DNA LoBind tube
- ☐ Adjust the volume to 47 µl with Nuclease-free water
- ☐ Mix thoroughly by pipetting up and down, or by flicking the tube
- ☐ Spin down briefly in a microfuge

In a 0.2 ml thin-walled PCR tube, mix the following:

- ☐ 47 µl gDNA from the previous step
- ☐ 1 µl DNA CS
- ☐ 3.5 µl NEBNext FFPE DNA Repair Buffer
- ☐ 2 µl NEBNext FFPE DNA Repair Mix
- ☐ 3.5 µl Ultra II End-prep Reaction Buffer
- ☐ 3 µl Ultra II End-prep Enzyme Mix

- ☐ Thoroughly mix the reaction by gently pipetting and briefly spinning down.
- ☐ Using a thermal cycler, incubate at 20°C for 5 minutes and 65°C for 5 minutes.
- ☐ Resuspend the AMPure XP Beads (AXP) by vortexing.
- ☐ Transfer the DNA sample to a clean 1.5 ml Eppendorf DNA LoBind tube.
- ☐ Add 60 µl of resuspended the AMPure XP Beads (AXP) to the end-prep reaction and mix by flicking the tube.
- ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.
- ☐ Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.
- ☐ Spin down the sample and pellet on a magnet for 10 minutes until the supernatant is clear and colourless. Keep the tube on the magnet, and pipette off the supernatant.
- ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.
- ☐ Repeat the previous step.

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
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| ☐ Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Allow to dry for ~30 seconds, but do not dry the pellet to the point of cracking.<br>☐ Remove the tube from the magnetic rack and resuspend the pellet in 61 µl Nuclease-free water. Incubate for 2 minutes at RT.<br>☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.<br>☐ Remove and retain 61 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. | |
| Quantify 1 µl of eluted sample using a Qubit fluorometer. | |
| Take forward the repaired and end-prepped DNA into the adapter ligation step. However, at this point it is also possible to store the sample at 4°C overnight. | |
| **Adapter ligation and clean-up** | |
| IMPORTANT<br>☐ Although the recommended third-party ligase is supplied with its own buffer, the ligation efficiency of the Ligation Adapter (LA) is higher when using the Ligation Buffer (LNB) supplied in the Ligation Sequencing Kit. | |
| ☐ Spin down the Ligation Adapter (LA) and Quick T4 Ligase, and place on ice.<br>☐ Thaw Ligation Buffer (LNB) at RT, spin down and mix by pipetting. Due to viscosity, vortexing this buffer is ineffective. Place on ice immediately after thawing and mixing.<br>☐ Thaw the Elution Buffer (EB) at RT and mix by vortexing. Then spin down and place on ice.<br>☐ Thaw the Long Fragment Buffer (LFB) at RT and mix by vortexing. Then spin down and place on ice.<br>In a 1.5 ml Eppendorf DNA LoBind tube, mix in the following order:<br>☐ 60 µl DNA sample from the previous step<br>☐ 25 µl Ligation Buffer (LNB)<br>☐ 10 µl NEBNext Quick T4 DNA Ligase<br>☐ 5 µl Ligation Adapter (LA)<br>☐ Thoroughly mix the reaction by gently pipetting and briefly spinning down.<br>☐ Incubate the reaction for 10 minutes at RT.<br>☐ Resuspend the AMPure XP Beads (AXP) by vortexing.<br>☐ Add 40 µl of resuspended AMPure XP Beads (AXP) to the reaction and mix by flicking the tube.<br>☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.<br>☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. | |

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| ☐ Wash the beads by adding 250 µl Long Fragment Buffer (LFB). Flick the beads to resuspend, spin down, then return the tube to the magnetic rack and allow the beads to pellet for at least 5 minutes. Remove the supernatant using a pipette and discard.<br>☐ Repeat the previous step.<br>☐ Spin down and place the tube back on the magnet. Pipette off any residual supernatant. Allow to dry for ~30 seconds, but do not dry the pellet to the point of cracking.<br>☐ Remove the tube from the magnetic rack and resuspend the pellet in 25 µl Elution Buffer (EB). Spin down and incubate for 10 minutes at 37°C.<br>☐ Pellet the beads on a magnet for 10 minutes until the eluate is clear and colourless.<br>☐ Remove and retain 25 µl of eluate containing the DNA library into a clean 1.5 ml Eppendorf DNA LoBind tube. | |
| Quantify 1 µl of eluted sample using a Qubit fluorometer. | |
| ☐ Split your library into three libraries of 300 ng (10-20 fmol) in 32 µl using Elution Buffer (EB).<br>IMPORTANT<br>☐ For libraries with an N50 of 30 kb, we recommend loading 300 ng (10-20 fmol) of your final library onto the R10.4.1 flow cell. | |
| The prepared library is used for loading into the flow cell. Store the library on ice or at 4°C until ready to load. | |
| **Priming and loading the PromethION Flow Cell** | |
| IMPORTANT<br>☐ This kit is only compatible with R10.4.1 flow cells (FLO-PRO114M). | |
| ☐ 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.<br>To prepare the flow cell priming mix, combine Flow Cell Tether (FCT) and Flow Cell Flush (FCF), as directed below. Mix by vortexing at RT.<br>☐ 1,170 µl Flow Cell Flush (FCF)<br>☐ 30 µl Flow Cell Tether (FCT) | |
| IMPORTANT<br>☐ 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. | |
| For PromethION 2 Solo, load the flow cell(s) as follows:<br>☐ Place the flow cell flat on the metal plate.<br>☐ Slide the flow cell into the docking port until the gold pins or green board cannot be seen. | |

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| For the PromethION 24/48, load the flow cell(s) into the docking ports:<br>☐ Line up the flow cell with the connector horizontally and vertically before smoothly inserting into position.<br>☐ Press down firmly onto the flow cell and ensure the latch engages and clicks into place. | |
| IMPORTANT<br>☐ 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. | |
| ☐ Slide the inlet port cover clockwise to open. | |
| IMPORTANT<br>☐ 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:<br>☐ Set a P1000 pipette tip to 200 µl.<br>☐ Insert the tip into the inlet port.<br>☐ Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip.<br>☐ Load 500 µl of the priming mix into the flow cell via the inlet port, avoiding the introduction of air bubbles. Wait five minutes. During this time, prepare the library for loading using the next steps in the protocol.<br>☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting. | |
| IMPORTANT<br>☐ 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 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:<br>☐ 100 µl Sequencing Buffer (SB)<br>☐ 68 µl Library Beads (LIB) thoroughly mixed before use, or Library Solution (LIS)<br>☐ 32 µl DNA library<br>☐ Complete the flow cell priming by slowly loading 500 µl of the priming mix into the inlet port.<br>☐ Mix the prepared library gently by pipetting up and down just prior to loading.<br>☐ Load 200 µl of library into the inlet port using a P1000 pipette.<br>☐ Close the valve to seal the inlet port. | |
| IMPORTANT<br>☐ 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:<br>☐ 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.<br>☐ Firmly press the light shield around the inlet port cover. The inlet port clip will click into place underneath the inlet port cover. | |

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| Close the PromethION lid when ready to start a sequencing run on MinKNOW. | |
| **Washing and reloading a PromethION Flow Cell** | |
| We recommend to wash the flow cell when ~25% starting pore count is remaining.<br><br>☐ Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.<br>☐ Thaw one tube of Wash Diluent (DIL) at RT.<br>☐ Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.<br><br>In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:<br>☐ 2 µl Wash Mix (WMX)<br>☐ 398 µl Wash Diluent (DIL)<br><br>☐ Mix well by pipetting, and place on ice. Do not vortex the tube.<br><br>☐ Pause the sequencing experiment in MinKNOW, and leave the flow cell in the device. | |
| IMPORTANT<br>☐ 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:<br>☐ Close the inlet port.<br>☐ Insert a P1000 pipette into a waste port and remove the waste buffer.<br><br>☐ Slide the inlet port cover clockwise to open the inlet port. | |
| IMPORTANT<br>☐ 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:<br>☐ Set a P1000 pipette to 200 µl<br>☐ Insert the tip into the inlet port<br>☐ Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip.<br><br>Slowly load 200 µl of the prepared flow cell wash mix into the inlet port, as follows:<br>☐ Using a P1000 pipette, take 200 µl of the flow cell wash mix<br>☐ Insert the pipette tip into the inlet port, ensuring there are no bubbles in the tip<br>☐ 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.<br>☐ Set a timer for a 5 minute incubation. | |

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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

IMPORTANT

☐ The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading the subsequent library. However, number of available pores will be reported after the next pore scan.

☐ 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 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)

☐ 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, draw back a small volume to remove any air bubbles:

- ☐ Set a P1000 pipette tip to 200 µl.
- ☐ Insert the tip into the inlet port.
- ☐ Turn the wheel until the dial shows 220-230 µl, or until you 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.

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
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| ☐ Close the inlet port and wait five minutes.<br><br>☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting. | |
| IMPORTANT<br>☐ 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 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:<br>☐ 100 µl Sequencing Buffer (SB)<br>☐ 68 µl Library Beads (LIB) thoroughly mixed before use, or Library Solution (LIS)<br>☐ 32 µl DNA library | |
| IMPORTANT<br>☐ 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:<br>☐ Ensure the inlet port is closed.<br>☐ Insert a P1000 pipette into a waste port and remove the waste buffer<br><br>☐ Slide the inlet port cover clockwise to open. | |
| IMPORTANT<br>☐ 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:<br>☐ Set a P1000 pipette tip to 200 µl.<br>☐ Insert the tip into the inlet port.<br>☐ Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip.<br><br>Slowly load 500 µl of the priming mix into the inlet port, as follows:<br>☐ Using a P1000 pipette, take 500 µl of the priming mix<br>☐ Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip<br>☐ 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<br>☐ 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:<br>☐ Close the inlet port.<br>☐ Insert a P1000 pipette into a waste port and remove the waste buffer.<br><br>☐ Slide the inlet port cover clockwise to open. | |

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| INSTRUCTIONS | NOTES/OBSERVATIONS |
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| IMPORTANT<br>☐ 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:<br>☐ Set a P1000 pipette tip to 200 µl.<br>☐ Insert the tip into the inlet port.<br>☐ Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip.<br>☐ Mix the prepared library gently by pipetting up and down just prior to loading.<br>☐ Load 200 µl of library into the inlet port using a P1000 pipette.<br>☐ Close the valve to seal the inlet port. | |
| IMPORTANT<br>☐ 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:<br>☐ 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.<br>☐ Firmly press the light shield around the inlet port cover. The inlet port clip will click into place underneath the inlet port cover.<br>☐ Close the PromethION lid when ready to start a sequencing run on MinKNOW. | |
| Perform the "Washing and reloading a PromethION flow cell" step twice for a total of three library loads (initial library load + two wash and reloads) to maximise data acquisition. | |
| **Flow cell reuse and returns** | |
| ☐ After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Flow Cell Wash Kit protocol and store the washed flow cell at 2-8°C.<br>☐ Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore. | |
| IMPORTANT<br>☐ If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in the online version of this protocol. | |