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

Before start checklist

Materials

- [ ] 3 µg high molecular weight human genomic DNA
- [ ] Sequence capture kit (e.g. Agilent SureSelect Human All Exon, Cat# 232866)
- [ ] Custom primer mix, 10 µM (IDT) - see below for sequences
- [ ] Ligation Sequencing Kit (SQK-LSK109)
- [ ] Flow Cell Priming Kit (EXP-FLP002)
- [ ] PCR Expansion (EXP-PCA001)

Consumables

- [ ] NEBNext dsDNA Fragmentase (M0348L)
- [ ] NEB Blunt/TA Ligase Master Mix (NEB, cat # M0367)
- [ ] NEBNext Ultra II End repair/dA-tailing Module (NEB, E7546)
- [ ] NEBNext Quick Ligation Module (NEB, E6056)
- [ ] Agencourt AMPure XP beads (Beckman Coulter, A63881)
- [ ] Cot-1 DNA (ThermoFisher Scientific 15279-011)
- [ ] Dynabeads MyOne Streptavidin T1 (ThermoFisher Scientific, 65601)
- [ ] Blocking oligo at 1 mM, sequence 5'-AGGTTAAACACCCAAGCAGACGCCGCAATATCAGCACCAACAGAAACAACC-3'
- [ ] LongAmp Taq 2X Master Mix (e.g. NEB, cat # M0287)
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] Freshly prepared 80% ethanol in nuclease-free water
- [ ] Freshly prepared 70% ethanol in nuclease-free water
- [ ] 0.5 M EDTA, pH 8 (Thermo Scientific, R1021)
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] 0.2 ml 96 well PCR plate

Equipment

- [ ] Hula mixer (gentle rotator mixer)
- [ ] Magnetic rack, suitable for 1.5 ml Eppendorf tubes
- [ ] Microfuge
- [ ] Vortex mixer
- [ ] Thermal cycler
- [ ] Ice bucket with ice
- [ ] Timer
- [ ] SpeedVac
- [ ] Pipettes and pipette tips P2, P10, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS

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INSTRUCTIONS	NOTES/OBSERVATIONS
DNA fragmentation	
Prepare the DNA in Nuclease-free water. ☐ Transfer 3 µg genomic DNA into a 1.5 ml Eppendorf DNA LoBind tube ☐ Adjust the volume to 16 µl with Nuclease-free water ☐ Mix thoroughly by flicking the tube to avoid unwanted shearing ☐ Spin down briefly in a microfuge In a 0.2 ml thin-walled PCR tube, mix the following: ☐ 16 µl DNA ☐ 2 µl 10x Fragmentase Reaction Buffer v2 ☐ Vortex the tube for 3 seconds, and spin down. ☐ Add 2 µl dsDNA Fragmentase to the tube. ☐ Incubate the reaction for 28 minutes at 37°C. ☐ Stop the fragmentation reaction by adding 5 µl of 0.5 M EDTA to the tube. ☐ Vortex the tube for 3 seconds, and spin down. ☐ Resuspend the AMPure XP beads by vortexing. ☐ Transfer the sample to a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 15 µl of resuspended AMPure XP beads to the reaction and mix by pipetting. ☐ Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT. ☐ Spin down the sample and pellet the beads on a magnet for 5 mins. ☐ Remove and retain the supernatant in a new 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 40 µl of resuspended AMPure XP beads to the reaction and mix by flicking the tube. ☐ Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT. ☐ Prepare 500 µl of fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet the beads on a magnet for 5 minutes. Keep the tube on the magnet until the eluate is clear and colourless, and pipette off the supernatant. ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ 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.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Remove the tube from the magnetic rack and resuspend pellet in 21 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless. ☐ Remove and retain 21 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Run a 1 µl aliquot on an Agilent Bioanalyzer to determine fragment length.	
Take the fragmented DNA in 20 µl into the end-prep step. However, at this point it is also possible to store the sample at 4°C overnight.	
End-prep	
In a 0.2 ml thin-walled PCR tube, mix the following: ☐ 20 µl Fragmented DNA ☐ 7 µl Ultra II End-prep reaction buffer ☐ 3 µl Ultra II End-prep enzyme mix ☐ 30 µl Nuclease-free water ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Using a thermal cycler, incubate at 20°C for 30 minutes and 65°C for 30 mins. ☐ Resuspend the AMPure XP beads by vortexing. ☐ Transfer the DNA sample to a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 60 µl of resuspended AMPure XP beads 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 80% ethanol in Nuclease-free water. ☐ 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. ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend pellet in 31 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute. ☐ Remove and retain 31 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Quantify 1 µl of end-prepped DNA using a Qubit fluorometer.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Take forward ~300 ng of end-prepped DNA in 30 µl into adapter ligation. However, at this point it is also possible to store the sample at 4°C overnight.	
Ligation of PCR adapters	
Add the reagents in the order given below, mixing by pipetting 10-20 times between each sequential addition: ☐ 30 µl End-prepped DNA ☐ 20 µl PCR Adapters (PCA) ☐ 50 µl NEB Blunt/TA Ligase Master Mix ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the reaction for 10 minutes at RT. ☐ Resuspend the AMPure XP beads by vortexing. ☐ Add 100 µl of resuspended AMPure XP beads to the end-prep reaction and mix by pipetting. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Prepare 500 µl of fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend pellet in 49 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless. ☐ Remove and retain 49 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Quantify 1 µl of adapted DNA using a Qubit fluorometer.	
Take forward 48 µl of the adapted DNA into the PCR reaction. However, at this point it is also possible to store the sample at 4°C overnight.	
PCR	
In a 0.2 ml thin-walled PCR tube mix the following: ☐ 50 µl LongAmp Taq 2X Master Mix ☐ 2 µl PRM Adapters (10 µM) ☐ 48 µl Template DNA	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Amplify using the following cycling conditions: ☐ Initial denaturation 3 mins @ 95 °C (1 cycle) ☐ Denaturation 20 secs @ 98 °C (6 (b) cycles) ☐ Annealing 15 secs (a) @ 62 °C (a) (6 (b) cycles) ☐ Extension 3 mins @ 65 °C (c) (6 (b) cycles) ☐ Final extension 3 mins @ 65 °C (1 cycle) ☐ Hold @ 4 °C ☐ Resuspend the AMPure XP beads by vortexing. ☐ Add 100 µl of the resuspended AMPure XP beads to the sample, and mix by flicking the tube. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Prepare 500 µl of fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend the pellet in 35 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless. ☐ Remove and retain 35 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Quantify 2 µl of amplified DNA using a Qubit fluorometer.	
Take forward 300–1000 ng amplified DNA into the hybridisation step. However, at this point it is also possible to store the sample at 4°C overnight.	
Hybridisation	
In a clean 1.5 ml Eppendorf DNA LoBind tube, mix the following: ☐ 300–1000 ng DNA library ☐ 5 µg Cot-1 DNA ☐ 1 µl Blocking oligo top The volume of the reaction can be variable, as the water is evaporated in step 2. After this, the DNA is reconstituted to a set volume. ☐ Evaporate the water in a SpeedVac at 45ºC for approximately 1 hour.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Reconstitute with Nuclease-free water to a final volume of 9 µl. Pipette up and down along the sides of the tube for optimal recovery. ☐ Mix thoroughly by vortexing and spin down for 1 minute. Move the 9 µl DNA library sample to a 0.2 µl thin-walled PCR tube, close the tube and incubate in the thermal cycler using the following program: ☐ Step 1 - Temperature: 95°C - Time: 5 min ☐ Step 2 - Temperature: 65°C - Time: 5 min ☐ Step 3 - Temperature: 65°C - Time: Hold You will now need to prepare the Hybridisation Buffer mix and the RNase Block ready to be combined with the Capture Library reagent from the SureSelect kit. This will then be combined with the adapted, amplified DNA sample. Once the sample tube is in the thermal cycler, mix the reagents in the table below to make the Hybridisation Buffer: ☐ 6.63 µl SureSelect Hyb 1 (orange cap or bottle) ☐ 0.27 µl SureSelect Hyb 2 (red cap) ☐ 2.65 µl SureSelect Hyb 3 (yellow cap or bottle) ☐ 3.45 µl SureSelect Hyb 4 (black cap or bottle) Dilute the SureSelect RNase Block (purple cap) in Nuclease-free water. Keep the mixture on ice. ☐ 2 µl 25% (1:3) Prepare the Capture Library Hybridisation Mix according to the table below. Only keep the mixture at RT until it is added to sample tube. ☐ 13 µl Hybridisation buffer mixture ☐ 2 µl 25% RNase Block solution ☐ 5 µl Capture library (red cap) ≥3 Mb	
IMPORTANT ☐ Do not keep solutions containing the Capture Library at RT for longer than 10 mins.	
☐ Keeping all reagents at 65°C, add 20 µl of the Capture Hybridisation Mix to the tube containing 9 µl adapted and amplified DNA sample. ☐ Mix by pipetting. ☐ Replace the cap on the tube.	
IMPORTANT ☐ The tube must be closed to minimise evaporation and avoid a negative impact on your results.	
☐ Incubate the tube for 16–24 hours at 65°C with a heated lid set at 105°C.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Take your sample forward into the next step.	
Pull-down	
☐ Warm the SureSelect Wash Buffer 2 at 65°C.	
☐ Resuspend the Dynabeads MyOne Streptavidin T1 magnetic beads by vortexing.	
☐ Add 50 µl of the beads to a fresh 1.5 ml Eppendorf DNA LoBind tube.	
☐ Add 200 µl of SureSelect Binding Buffer to the beads.	
☐ Mix by pipetting.	
☐ Place on a magnetic rack, allow beads to pellet and pipette off supernatant.	
☐ Repeat steps 4–6 twice more for a total of three washes.	
☐ Resuspend the beads in 200 µl of SureSelect Binding Buffer.	
☐ Keep the hybridisation tube at 65°C. Using a multichannel pipette, transfer the whole volume (~25–29 µl) of the hybridisation mixture from the 65°C reaction to the tube containing 200 µl of washed streptavidin beads.	
☐ Pipette up and down until beads are fully resuspended.	
☐ Incubate the tube on a Hula mixer for 30 mins at RT. Make sure the sample is mixing in the tube.	
☐ 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.	
☐ Resuspend the beads in 200 µl of SureSelect Wash Buffer 1.	
☐ Pipette up and down until beads are fully resuspended.	
☐ Incubate the reaction for 15 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.	
☐ Resuspend the beads in 200 µl of Wash Buffer 2 pre-warmed at 65°C.	
☐ Pipette up and down until beads are fully resuspended.	
☐ Transfer the sample to a 0.2 ml thin-walled PCR tube.	
☐ Incubate the tube for 10 minutes at 65°C in the thermal cycler.	
☐ Transfer the sample to a clean 1.5 ml Eppendorf DNA LoBind tube.	
☐ Place on a magnetic rack, allow beads to pellet and pipette off supernatant.	
☐ Repeat the wash steps 17–22 twice more for a total of three washes. Make sure all of the wash buffer has been removed during the final wash.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Add 96 µl of Nuclease-free water to the sample, and pipette up and down to resuspend the beads. Keep the sample on ice. Captured DNA remains on the streptavidin beads during the post-capture amplification step.	
Elution and amplification of DNA	
Custom primer mix sequences ☐ Split the sample into 2x 48 µl aliquots, and prepare the following reaction in duplicate. In a 0.2 ml thin-walled PCR tube, mix the following: ☐ 50 µl LongAmp Taq 2x Master Mix ☐ 2 µl Custom primer mix ☐ 48 µl Template DNA Amplify using the following cycling conditions: ☐ Initial denaturation 3 mins @ 95°C (1 cycle) ☐ Denaturation 20 secs @ 98°C (14 (b) cycles) ☐ Annealing 15 secs (a) @ 62°C (a) (14 (b) cycles) ☐ Extension 3 mins @ 65°C (c) (14 (b) cycles) ☐ Final extension 3 mins @ 65°C (1 cycle) ☐ Hold @ 4°C ☐ Place the amplified sample on a magnetic rack. Once the solution is clear, transfer the supernatant into a clean 1.5 ml Eppendorf DNA Lo-Bind tube. The beads can now be discarded. ☐ Resuspend the AMPure XP beads by vortexing. ☐ Add 180 µl of resuspended AMPure XP beads to the reaction and mix by pipetting. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Prepare sufficient fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. ☐ Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend pellet in 25 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Remove and retain 25 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. Pool the two samples together to yield 50 µl eluted sample.	
Quantify 1 µl of eluted sample using a Qubit fluorometer.	
☐ Run a 1 µl aliquot on an Agilent Bioanalyzer to determine fragment length.	
End-prep	
Perform end-repair and dA-tailing as follows: ☐ 48 µl DNA ☐ 7 µl Ultra II End-prep reaction buffer ☐ 3 µl Ultra II End-prep enzyme mix ☐ 2 µl Nuclease-free water ☐ Mix gently by flicking the tube, and spin down. ☐ Transfer the sample to a 0.2 ml thin-walled PCR tube. ☐ Using a thermal cycler, incubate at 20°C for 30 minutes and 65°C for 30 mins. ☐ Resuspend the AMPure XP beads by vortexing. ☐ Transfer the sample to a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 60 µl of resuspended AMPure XP beads to the end-prep reaction and mix by pipetting. ☐ 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. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. ☐ 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. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend pellet in 31 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless. ☐ Remove and retain 31 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Quantify 1 µl of end-prepped DNA using a Qubit fluorometer - recovery aim >300 ng.	
Take forward approximately 300 ng of end-prepped DNA in 30 µl into adapter ligation.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Adapter ligation and clean-up	
IMPORTANT ☐ Although the recommended third-party ligase is supplied with its own buffer, the ligation efficiency of Adapter Mix (AMX) is higher when using Ligation Buffer supplied within the Ligation Sequencing Kit.	
☐ Spin down the Adapter Mix (AMX) and Quick T4 Ligase, and place on ice. ☐ 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. ☐ Thaw the Elution Buffer (EB) at RT and mix by vortexing. Then spin down and place on ice. ☐ Thaw one tube of Short Fragment Buffer (SFB) at RT and mix by vortexing. Then spin down and place on ice. In a 1.5 ml Eppendorf DNA LoBind tube, mix in the following order: ☐ 30 µl DNA sample from the previous step ☐ 30 µl Nuclease-free water ☐ 25 µl Ligation Buffer (LNB) ☐ 10 µl NEBNext Quick T4 DNA Ligase ☐ 5 µl Adapter Mix (AMX) ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the reaction for 10 minutes at RT.	
IMPORTANT ☐ If you have omitted the AMPure purification step after DNA repair and end-prep, do not incubate the reaction for longer than 10 minutes.	
☐ Resuspend the AMPure XP beads by vortexing. ☐ Add 40 µl of resuspended AMPure XP beads to the reaction and mix by flicking the tube. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless. ☐ Wash the beads by adding 250 µl Short Fragment Buffer (SFB) - do NOT use Long Fragment buffer (LFB). Flick the beads to resuspend, then return the tube to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard. ☐ Repeat the previous step. ☐ 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. ☐ Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB). Spin down and incubate for 10 minutes at RT. For high molecular weight DNA, incubating at 37°C can improve the recovery of long fragments.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute. ☐ Remove and retain 15 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.	
☐ Use the average fragment size determined at the end of the "Elution and amplification of DNA" step to calculate the molarity of the sample. ☐ Take 50 fmol of library and make up the volume to 12 µl with EB.	
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 SpotON flow cell	
☐ Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FB) at RT before mixing the reagents by vortexing, and spin down at RT. ☐ To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) directly to the tube of thawed and mixed Flush Buffer (FB), and mix by vortexing at RT. ☐ Open the MinION Mk1B lid and slide the flow cell under the clip. ☐ 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 ☐ 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. During this time, prepare the library for loading by following the steps below. ☐ Thoroughly mix the contents of the Loading Beads (LB) by pipetting.	
IMPORTANT ☐ The Loading Beads (LB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
In a new tube, prepare the library for loading as follows: ☐ 37.5 µl Sequencing Buffer (SQB) ☐ 25.5 µl Loading Beads (LB), mixed immediately before use ☐ 12 µl DNA library 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 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 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.	
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.	
You are now ready to start your sequencing experiment in MinKNOW.	
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. ☐ Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore.	
IMPORTANT ☐ 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.	