1D Genomic DNA by ligation (SQK-LSK108)

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

Before start checklist

- [ ] Ligation Sequencing Kit 1D (SQK-LSK108)
- [ ] Library Loading Bead Kit (EXP-LLB001)
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Magnetic rack
- [ ] Microfuge
- [ ] Vortex mixer
- [ ] Heating block at 37 °C capable of taking 1.5 ml tubes
- [ ] Thermal cycler at 20 °C and 65 °C
- [ ] Ice bucket with ice
- [ ] Timer
- [ ] Pipettes P2, P10, P20, P100, P200, P1000
- [ ] Pipette tips P2, P10, P20, P100, P200, P1000
- [ ] Pipettes and pipette tips
- [ ] Agencourt AMPure XP beads
- [ ] NEBNext FFPE Repair Mix (M6630)
- [ ] NEBNext End repair / dA-tailing Module (E7546)
- [ ] NEB Blunt/TA Ligase Master Mix (M0367)
- [ ] Covaris g-TUBE
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] Nuclease-free water
- [ ] 10 mM Tris-HCl pH 8.5
- [ ] Freshly prepared 70% ethanol in nuclease-free water

INSTRUCTIONS	NOTES/OBSERVATIONS
Preparing input DNA	
Prepare the DNA in Nuclease-free water. ☐ Transfer 1-1.5 µg genomic DNA into a DNA LoBind tube ☐ Adjust the volume to 46 µl with Nuclease-free water ☐ Mix thoroughly by inversion avoiding unwanted shearing ☐ Spin down briefly in a microfuge ☐ Record the quality, quantity and size of the DNA.	
IMPORTANT Criteria for input DNA ☐ Purity as measured using Nanodrop - OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2 ☐ Average fragment size, as measured by pulse-field, or low percentage agarose gel analysis >30 kb ☐ Input mass, as measured by Qubit - 1 µg (~ 1.5 µg if carrying out a DNA repair step) ☐ No detergents or surfactants in the buffer	
Check your flow cell	
☐ Set up the MinION, Flow Cell and host computer Once successfully plugged in, you will see a light and hear the fan.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Open the MinKNOW GUI from the desktop icon and establish a local or remote connection. ☐ If running a MinION on the same host computer, plug the MinION into the computer. When the connection name appears under the Local tab, click Connect. ☐ If running a MinION on a remote computer, first enter the name or IP address of the remote host under Remote and click Connect. ☐ Plug a MinION and Flow Cell into the remote computer; the connection IDs will be displayed under MinION Connection and Flowcell Connection. Enter the SampleID and FlowcellID being used, and click Submit. ☐ Once a MinION and Flow Cell are connected, a Label Experiment dialogue box appears. ☐ Click into the Sample ID box and name your sample using free text in alphanumeric format only, deleting any default Sample_ID that is present. Warning: SampleID should not contain any personally identifiable information. ☐ Click into the FlowcellID box and enter the Flow Cell ID, which is the code found on a sticker on the top side of a Flow Cell. ☐ Select the Platform QC script under Choose Operation, and start the script using the Execute button. ☐ Check the number of active pores available for the experiment, reported in the message panel or in notifications when the check is complete.	
Flow cell check complete.	
DNA fragmentation (optional)	
OPTIONAL ☐ Transfer 1-1.5 µg genomic DNA in 46 µl to the Covaris g-TUBE. Spin the g-TUBE for 1 minute at RT at the speed for the fragment size required. ☐ Spin the g-TUBE for 1 minute ☐ Remove and check all the DNA has passed through the g-TUBE ☐ If DNA remains in the upper chamber, spin again for 1 minute at the same speed Invert the g-TUBE and spin again for 1 minute to collect the fragmented DNA. ☐ Remove g-TUBE, invert the tube and replace into the centrifuge ☐ Spin the g-TUBE for 1 minute ☐ Remove and check the DNA has passed into the lower chamber ☐ If DNA remains in the upper chamber, spin again for 1 minute ☐ Remove g-TUBE ☐ Transfer the 46 µl fragmented DNA to a clean 1.5 ml Eppendorf DNA LoBind tube.	
Analyse 1 µl of the fragmented DNA for fragment size, quantity and quality.	
1-1.5 µg fragmented DNA in 45 µl is taken into the next step.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
DNA repair (optional)	
OPTIONAL Perform FFPE DNA repair treatment using NEB M6630. ☐ 45µl 1-1.5 µg fragmented** DNA ☐ 8.5 µl Nuclease-free water ☐ 6.5 µl FFPE Repair Buffer ☐ 2 µl FFPE Repair Mix ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the reaction for 15 minutes at 20 °C. ☐ Prepare the AMPure XP beads for use; resuspend by vortexing. ☐ Add 62 µl of the resuspended beads to the FFPE-repair reaction and mix gently 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. Keep the tube on the magnet, and pipette off the supernatant. ☐ Keep on magnet, wash beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the 70% ethanol using a pipette and discard. Repeat. ☐ Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Briefly allow to dry. ☐ Remove the tube from the magnetic rack and resuspend pellet in 46 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet beads on magnet until the eluate is clear and colourless. ☐ Remove and retain 46 µl of eluate in a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Quantify 1 µl of fragmented and repaired DNA using a Qubit fluorometer - recovery aim > 1 µg.	
Take 1 µg of FFPE repaired DNA in 45 µl into End-prep.	
End-prep	
Perform end repair and dA-tailing of fragmented DNA as follows: ☐ 45 µl ~1 µg DNA (fragmented genomic DNA, amplicon or cDNA) ☐ 7 µl Ultra II End-prep reaction buffer ☐ 3 µl Ultra II End-prep enzyme mix ☐ 5 µl Nuclease-free water ☐ Mix gently by flicking the tube, and spin down. ☐ Transfer the sample to a 0.2 ml PCR tube, and incubate for 5 minutes at 20 °C and 5 minutes at 65 °C using the thermal cycler. ☐ Prepare the AMPure XP beads for use; resuspend by vortexing.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Transfer the sample to a 1.5 ml DNA LoBind Eppendorf 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. ☐ Keep on magnet, wash beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the 70% ethanol using a pipette and discard. Repeat. ☐ Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Briefly allow to dry. ☐ Remove the tube from the magnetic rack and resuspend pellet in 31 µl Nuclease-free water. Incubate for 2 minutes at RT. ☐ Pellet beads on 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 > 700 ng.	
Take forward approximately 700 ng of end-prepped DNA in 30 µl into adapter ligation.	
Adapter ligation	
Thaw and prepare the kit reagents as follows: ☐ ABB Buffer (ABB) at RT ☐ Elution Buffer (ELB) at RT ☐ Adapter Mix 1D (AMX1D) on ice ☐ Running Buffer with Fuel Mix (RBF) on ice ☐ Blunt/TA Ligation Master Mix on ice Check the contents of each tube are clear of any precipitate and are thoroughly mixed before setting up the reaction. ☐ Mix the contents of each tube by flicking ☐ Check that there is no precipitate present (DTT in the Blunt/TA Master Mix can sometimes form a precipitate) ☐ Spin down briefly before accurately pipetting the contents in the reaction Taking the end-prepped DNA, perform adapter ligation as follows, mixing by flicking the tube between each sequential addition. ☐ 30 µl End-prepped DNA ☐ 20 µl Adapter Mix ☐ 50 µl Blunt/TA Ligation Master Mix ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the reaction for 10 minutes at RT.	AMX 1D

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INSTRUCTIONS	NOTES/OBSERVATIONS
AMPure XP bead binding	
☐ Prepare the AMPure XP beads for use; resuspend by vortexing. ☐ Add 40 µl of resuspended AMPure XP beads to the adapter ligation reaction from the previous step and mix by pipetting. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Place on magnetic rack, allow beads to pellet and pipette off supernatant. ☐ Add 140 µl of the ABB buffer to the beads. Close the tube lid, and resuspend the beads by flicking the tube. Return the tube to the magnetic rack, allow beads to pellet and pipette off the supernatant. Repeat. ☐ Remove the tube from the magnetic rack and resuspend pellet in 15 µl Elution Buffer. Incubate for 10 minutes at RT. ☐ Pellet beads on magnet until the eluate is clear and colourless. Remove and retain 15 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Remove and retain the eluate which contains the DNA library in a clean 1.5 ml Eppendorf DNA LoBind tube ☐ Dispose of the pelleted beads	
The prepared library is used for loading into the MinION Flow Cell. Store the library on ice until ready to load.	
☐ Quantify 1 µl of fragmented and repaired DNA using a Qubit fluorometer - recovery aim ~430 ng.	

Before sequencing checklist

☐ Prepared library on ice	☐ Computer set up to run MinKNOW	☐ Hardware check complete
☐ Sequencing device connected to computer with SpotON Flow Cell inserted	☐ Desktop Agent set up (if applicable)	☐ Flow cell check complete

Priming and loading the SpotON Flow Cell	
IMPORTANT ☐ Thoroughly mix the contents of the RBF tube by vortexing or pipetting, and spin down briefly.	RBF LLB
☐ Flip back the MinION lid and slide the priming port cover clockwise so that the priming port is visible.	
IMPORTANT ☐ Care must be taken when drawing back buffer from the flow cell. The array of pores must be covered by buffer at all times. Removing more than 20-30 µl risks damaging the pores in the array.	
After opening the priming port, check for small bubble under the cover. Draw back a small volume to remove any bubble (a few µls): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the priming 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	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Prepare the flow cell priming mix in a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ 576 µl RBF ☐ 624 µl Nuclease-free water ☐ Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for 5 minutes. ☐ Thoroughly mix the contents of the RBF and LLB tubes by pipetting. In a new tube, prepare the library for loading as follows: ☐ 35.0 µl RBF ☐ 2.5 µl Nuclease-free water ☐ 25.5 µl LLB ☐ 12.0 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 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 75 µl of sample 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, close the priming port and replace the MinION lid.	
Starting a sequencing run	
☐ Double–click the MinKNOW icon located on the desktop to open the MinKNOW GUI. ☐ Wait for the MinKNOW GUI to open ☐ Select the local MinION, and click Connect. Enter the SampleID and FlowcellID being used, and click Submit. ☐ Once a MinION and Flow Cell are connected, a Label Experiment dialogue box appears. ☐ Click into the Sample ID box and name your sample using free text in alphanumeric format only, deleting any default Sample_ID that is present. Warning: SampleID should not contain any personally identifiable information. ☐ Click into the FlowcellID box and enter the Flow Cell ID, which is the code found on a sticker on the top side of a Flow Cell. Select the appropriate protocol script ☐ Experiment type: Choose Sequencing Run under "Choose Operation" ☐ Flow Cell product code: Choose the Flow Cell type under "Flow cell product code" ☐ Sequencing kit: Choose SQK-LSK108 under Sequencing Kit ☐ Choose whether or not live basecalling is enabled ☐ The most appropriate script will appear in the drop-down menu.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Start the script using the Execute button at the bottom of the Connections page. Allow the script to run to completion. ☐ The MinKNOW Experiment page will indicate the progression of the script ☐ Monitor messages in the Message panel in the MinKNOW GUI The basecalled read files are stored in :\data\reads	
Progression of MinKNOW protocol script	
Check the number of active pores reported in the MUX scan are similar (within 10-15%) to those reported at the end of the Platform QC ☐ If there is a significant reduction in the numbers, restart MinKNOW. ☐ If the numbers are still significantly different, close down the host computer and reboot. ☐ When the numbers are similar to those reported at the end of the Platform QC, restart the experiment on the Connection page. There is no need to load any additional library after restart. ☐ Check the heatsink temperature is approximately 34 °C. ☐ Monitor the development of the read length histogram. ☐ Check pore occupancy by looking at the panel at the top of the Status or Physical Layout views.	
Further analysis with EPI2ME (optional)	
OPTIONAL ☐ Open the Desktop Agent using the desktop shortcut. ☐ Click on the New Workflow tab in the Desktop Agent and select the workflow to be used in the analysis. ☐ Check the correct settings are selected in the Desktop Agent. ☐ Click "Start Run" to start data analysis. ☐ Follow the progression of upload and download of read files in the Desktop Agent. Click on VIEW REPORT. ☐ Click on VIEW REPORT to navigate to the EPI2ME website, this can be done at any point during data exchange ☐ Return to the Desktop Agent to see progression of the exchange ☐ When the upload and download numbers are the same, the data exchange is complete. The processed reads will be in downloads folder in the selected location on the host computer.	
Close down MinKNOW and the Desktop Agent	
☐ Quit Desktop Agent using the close x. ☐ Quit MinKNOW by closing down the web GUI. ☐ Disconnect the MinION.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Prepare the flow cell for re-use or return to Oxford Nanopore.	
☐ If you would like to reuse the flow cell, follow the Wash Kit instructions and store the washed flow cell at 2-8 °C, OR ☐ Follow the returns procedure by washing out the MinION Flow Cell ready to send back to Oxford Nanopore.	