

1D Genomic DNA by ligation (SQK-LSK108)

Version: GDE_9002_v108_revT_18Oct2016
Last update: 23/10/2017



Flow Cell Number:

DNA Samples:

Before start checklist

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

INSTRUCTIONS	NOTES/OBSERVATIONS
Preparing input DNA	
<p>Prepare the DNA in Nuclease-free water.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Transfer 1-1.5 µg genomic DNA into a DNA LoBind tube <input type="checkbox"/> Adjust the volume to 46 µl with Nuclease-free water <input type="checkbox"/> Mix thoroughly by inversion avoiding unwanted shearing <input type="checkbox"/> Spin down briefly in a microfuge <p><input type="checkbox"/> Record the quality, quantity and size of the DNA.</p>	
<p>IMPORTANT</p> <p>Criteria for input DNA</p> <ul style="list-style-type: none"> <input type="checkbox"/> Purity as measured using Nanodrop - OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2 <input type="checkbox"/> Average fragment size, as measured by pulse-field, or low percentage agarose gel analysis >30 kb <input type="checkbox"/> Input mass, as measured by Qubit - 1 µg (~ 1.5 µg if carrying out a DNA repair step) <input type="checkbox"/> No detergents or surfactants in the buffer 	
Check your flow cell	
<ul style="list-style-type: none"> <input type="checkbox"/> Set up the MinION, Flow Cell and host computer <p>Once successfully plugged in, you will see a light and hear the fan.</p>	

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<p>Open the MinKNOW GUI from the desktop icon and establish a local or remote connection.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 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. <input type="checkbox"/> If running a MinION on a remote computer, first enter the name or IP address of the remote host under Remote and click Connect. <input type="checkbox"/> Plug a MinION and Flow Cell into the remote computer; the connection IDs will be displayed under MinION Connection and Flowcell Connection. <p>Enter the SampleID and FlowcellID being used, and click Submit.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Once a MinION and Flow Cell are connected, a Label Experiment dialogue box appears. <input type="checkbox"/> 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. <input type="checkbox"/> 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. <ul style="list-style-type: none"> <input type="checkbox"/> Select the Platform QC script under Choose Operation, and start the script using the Execute button. <input type="checkbox"/> 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)	
<p>OPTIONAL</p> <ul style="list-style-type: none"> <input type="checkbox"/> Transfer 1-1.5 µg genomic DNA in 46 µl to the Covaris g-TUBE. <p>Spin the g-TUBE for 1 minute at RT at the speed for the fragment size required.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Spin the g-TUBE for 1 minute <input type="checkbox"/> Remove and check all the DNA has passed through the g-TUBE <input type="checkbox"/> If DNA remains in the upper chamber, spin again for 1 minute at the same speed <p>Invert the g-TUBE and spin again for 1 minute to collect the fragmented DNA.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Remove g-TUBE, invert the tube and replace into the centrifuge <input type="checkbox"/> Spin the g-TUBE for 1 minute <input type="checkbox"/> Remove and check the DNA has passed into the lower chamber <input type="checkbox"/> If DNA remains in the upper chamber, spin again for 1 minute <input type="checkbox"/> Remove g-TUBE <ul style="list-style-type: none"> <input type="checkbox"/> 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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<p>DNA repair (optional)</p> <p>OPTIONAL</p> <p>Perform FFPE DNA repair treatment using NEB M6630.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 45 µl 1-1.5 µg fragmented** DNA <input type="checkbox"/> 8.5 µl Nuclease-free water <input type="checkbox"/> 6.5 µl FFPE Repair Buffer <input type="checkbox"/> 2 µl FFPE Repair Mix <ul style="list-style-type: none"> <input type="checkbox"/> Mix gently by flicking the tube, and spin down. <input type="checkbox"/> Incubate the reaction for 15 minutes at 20 °C. <input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing. <input type="checkbox"/> Add 62 µl of the resuspended beads to the FFPE-repair reaction and mix gently by flicking the tube. <input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. <input type="checkbox"/> Prepare 500 µl of fresh 70% ethanol in Nuclease-free water. <input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant. <input type="checkbox"/> 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. <input type="checkbox"/> Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Briefly allow to dry. <input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 46 µl Nuclease-free water. Incubate for 2 minutes at RT. <input type="checkbox"/> Pellet beads on magnet until the eluate is clear and colourless. <input type="checkbox"/> Remove and retain 46 µl of eluate in a clean 1.5 ml Eppendorf DNA LoBind tube. <input type="checkbox"/> Quantify 1 µl of fragmented and repaired DNA using a Qubit fluorometer - recovery aim > 1 µg. 	
<p>Take 1 µg of FFPE repaired DNA in 45 µl into End-prep.</p>	
<p>End-prep</p> <p>Perform end repair and dA-tailing of fragmented DNA as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 45 µl ~1 µg DNA (fragmented genomic DNA, amplicon or cDNA) <input type="checkbox"/> 7 µl Ultra II End-prep reaction buffer <input type="checkbox"/> 3 µl Ultra II End-prep enzyme mix <input type="checkbox"/> 5 µl Nuclease-free water <ul style="list-style-type: none"> <input type="checkbox"/> Mix gently by flicking the tube, and spin down. <input type="checkbox"/> 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. <input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing. 	


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<ul style="list-style-type: none"> <input type="checkbox"/> Transfer the sample to a 1.5 ml DNA LoBind Eppendorf tube. <input type="checkbox"/> Add 60 µl of resuspended AMPure XP beads to the end-prep reaction and mix by pipetting. <input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. <input type="checkbox"/> Prepare 500 µl of fresh 70% ethanol in Nuclease-free water. <input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant. <input type="checkbox"/> 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. <input type="checkbox"/> Spin down and place the tube back on the magnet. Pipette off any residual ethanol. Briefly allow to dry. <input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 31 µl Nuclease-free water. Incubate for 2 minutes at RT. <input type="checkbox"/> Pellet beads on magnet until the eluate is clear and colourless. <input type="checkbox"/> Remove and retain 31 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. <input type="checkbox"/> 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	
<p>Thaw and prepare the kit reagents as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> ABB Buffer (ABB) at RT <input type="checkbox"/> Elution Buffer (ELB) at RT <input type="checkbox"/> Adapter Mix 1D (AMX1D) on ice <input type="checkbox"/> Running Buffer with Fuel Mix (RBF) on ice <input type="checkbox"/> Blunt/TA Ligation Master Mix on ice <p>Check the contents of each tube are clear of any precipitate and are thoroughly mixed before setting up the reaction.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Mix the contents of each tube by flicking <input type="checkbox"/> Check that there is no precipitate present (DTT in the Blunt/TA Master Mix can sometimes form a precipitate) <input type="checkbox"/> Spin down briefly before accurately pipetting the contents in the reaction <p>Taking the end-prepped DNA, perform adapter ligation as follows, mixing by flicking the tube between each sequential addition.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 30 µl End-prepped DNA <input type="checkbox"/> 20 µl Adapter Mix <input type="checkbox"/> 50 µl Blunt/TA Ligation Master Mix <ul style="list-style-type: none"> <input type="checkbox"/> Mix gently by flicking the tube, and spin down. <input type="checkbox"/> Incubate the reaction for 10 minutes at RT. 	<div style="text-align: center;">  </div>



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AMPure XP bead binding	
<input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing. <input type="checkbox"/> Add 40 µl of resuspended AMPure XP beads to the adapter ligation reaction from the previous step and mix by pipetting. <input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. <input type="checkbox"/> Place on magnetic rack, allow beads to pellet and pipette off supernatant. <input type="checkbox"/> 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. <input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 15 µl Elution Buffer. Incubate for 10 minutes at RT. <input type="checkbox"/> 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. <input type="checkbox"/> Remove and retain the eluate which contains the DNA library in a clean 1.5 ml Eppendorf DNA LoBind tube <input type="checkbox"/> 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.	
<input type="checkbox"/> Quantify 1 µl of fragmented and repaired DNA using a Qubit fluorometer - recovery aim ~430 ng.	
Before sequencing checklist	
<div> <input type="checkbox"/> Prepared library on ice <input type="checkbox"/> Computer set up to run MinKNOW <input type="checkbox"/> Hardware check complete </div> <div> <input type="checkbox"/> Sequencing device connected to computer with SpotON Flow Cell inserted <input type="checkbox"/> Desktop Agent set up (if applicable) <input type="checkbox"/> Flow cell check complete </div>	
Priming and loading the SpotON Flow Cell	
IMPORTANT <input type="checkbox"/> Thoroughly mix the contents of the RBF tube by vortexing or pipetting, and spin down briefly.	 
<input type="checkbox"/> Flip back the MinION lid and slide the priming port cover clockwise so that the priming port is visible.	
IMPORTANT <input type="checkbox"/> 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): <input type="checkbox"/> Set a P1000 pipette to 200 µl <input type="checkbox"/> Insert the tip into the priming port <input type="checkbox"/> 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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<p>Prepare the flow cell priming mix in a clean 1.5 ml Eppendorf DNA LoBind tube.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 576 µl RBF <input type="checkbox"/> 624 µl Nuclease-free water <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Thoroughly mix the contents of the RBF and LLB tubes by pipetting.</p> <p>In a new tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 35.0 µl RBF <input type="checkbox"/> 2.5 µl Nuclease-free water <input type="checkbox"/> 25.5 µl LLB <input type="checkbox"/> 12.0 µl DNA library <p>Complete the flow cell priming:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Gently lift the SpotON sample port cover to make the SpotON sample port accessible. <input type="checkbox"/> 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. <input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading. <input type="checkbox"/> 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. <input type="checkbox"/> Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION lid. 	
<p>Starting a sequencing run</p>	
<ul style="list-style-type: none"> <input type="checkbox"/> Double-click the MinKNOW icon located on the desktop to open the MinKNOW GUI. <input type="checkbox"/> Wait for the MinKNOW GUI to open <input type="checkbox"/> Select the local MinION, and click Connect. <p>Enter the SampleID and FlowcellID being used, and click Submit.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Once a MinION and Flow Cell are connected, a Label Experiment dialogue box appears. <input type="checkbox"/> 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. <input type="checkbox"/> 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. <p>Select the appropriate protocol script</p> <ul style="list-style-type: none"> <input type="checkbox"/> Experiment type: Choose Sequencing Run under "Choose Operation" <input type="checkbox"/> Flow Cell product code: Choose the Flow Cell type under "Flow cell product code" <input type="checkbox"/> Sequencing kit: Choose SQK-LSK108 under Sequencing Kit <input type="checkbox"/> Choose whether or not live basecalling is enabled <input type="checkbox"/> The most appropriate script will appear in the drop-down menu. 	

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<input type="checkbox"/> Start the script using the Execute button at the bottom of the Connections page. Allow the script to run to completion. <input type="checkbox"/> The MinKNOW Experiment page will indicate the progression of the script <input type="checkbox"/> 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 <input type="checkbox"/> If there is a significant reduction in the numbers, restart MinKNOW. <input type="checkbox"/> If the numbers are still significantly different, close down the host computer and reboot. <input type="checkbox"/> 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. <input type="checkbox"/> Check the heatsink temperature is approximately 34 °C. <input type="checkbox"/> Monitor the development of the read length histogram. <input type="checkbox"/> Check pore occupancy by looking at the panel at the top of the Status or Physical Layout views.	
Further analysis with EPI2ME (optional)	
OPTIONAL <input type="checkbox"/> Open the Desktop Agent using the desktop shortcut. <input type="checkbox"/> Click on the New Workflow tab in the Desktop Agent and select the workflow to be used in the analysis. <input type="checkbox"/> Check the correct settings are selected in the Desktop Agent. <input type="checkbox"/> Click "Start Run" to start data analysis. <input type="checkbox"/> Follow the progression of upload and download of read files in the Desktop Agent, along with network speed. Click on VIEW REPORT. <input type="checkbox"/> Click on VIEW REPORT to navigate to the EPI2ME website, this can be done at any point during data exchange <input type="checkbox"/> Return to the Desktop Agent to see progression of the exchange <input type="checkbox"/> 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	
<input type="checkbox"/> Quit Desktop Agent using the close x. <input type="checkbox"/> Quit MinKNOW by closing down the web GUI. <input type="checkbox"/> Disconnect the MinION.	

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Prepare the flow cell for re-use or return to Oxford Nanopore.	
<input type="checkbox"/> 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	
<input type="checkbox"/> Follow the returns procedure by washing out the MinION Flow Cell ready to send back to Oxford Nanopore.	