

1D Lambda Control Experiment (SQK-LSK109)

Version: CDE_9062_v109_revA_23May2018
 Last update: 23/05/2018



Flow Cell Number:

DNA Samples:

Before start checklist		
Materials	Consumables	Equipment
<input type="checkbox"/> Ligation Sequencing Kit 1D (SQK-LSK109)	<input type="checkbox"/> Agencourt AMPure XP beads	<input type="checkbox"/> Hula mixer (gentle rotator mixer)
<input type="checkbox"/> Flow Cell Priming Kit (EXP-FLP001)	<input type="checkbox"/> NEBNext FFPE Repair Mix (M6630)	<input type="checkbox"/> Magnetic rack
	<input type="checkbox"/> NEBNext End repair / dA-tailing Module (E7546)	<input type="checkbox"/> Microfuge
	<input type="checkbox"/> NEBNext Quick Ligation Module (E6056)	<input type="checkbox"/> Vortex mixer
	<input type="checkbox"/> 1.5 ml Eppendorf DNA LoBind tubes	<input type="checkbox"/> Thermal cycler at 20° C and 65° C
	<input type="checkbox"/> 0.2 ml thin-walled PCR tubes	<input type="checkbox"/> Ice bucket with ice
	<input type="checkbox"/> Nuclease-free water (e.g. ThermoFisher, cat # AM9937)	<input type="checkbox"/> Timer
	<input type="checkbox"/> Freshly prepared 70% ethanol in nuclease-free water	<input type="checkbox"/> Pipettes and pipette tips P2, P10, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
<p>Check your flow cell</p> <p><input type="checkbox"/> Set up the MinION, flow cell and host computer</p> <p>Once successfully plugged in, you will see a light and hear the fan.</p> <p>Open the MinkNOW GUI from the desktop icon and establish a local or remote connection.</p> <p><input type="checkbox"/> If running a MinION on the same host computer, plug the MinION into the computer.</p> <p><input type="checkbox"/> If running a MinION on a remote computer, first enter the name or IP address of the remote host under Connect to a remote computer (if running from the Connection page), or Connections (if running from the homepage) and click Connect.</p> <p><input type="checkbox"/> Choose the flow cell type from the selector box. Then mark the flow cell as "Selected":</p> <p>Click "Check flow cells" at the bottom of the screen.</p> <p><input type="checkbox"/> R9.4.1 FLO-MIN106</p> <p><input type="checkbox"/> R9.5.1 FLO-MIN107</p> <p><input type="checkbox"/> Click "Start test".</p> <p><input type="checkbox"/> Check the number of active pores available for the experiment, reported in the System History panel when the check is complete.</p>	
Flow cell check complete.	

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<p>DNA repair and end-prep</p> <p><input type="checkbox"/> Thaw DNA CS (DCS) and Lambda DNA (LMD) at RT, spin down, mix gently by pipetting, and place on ice.</p> <p><input type="checkbox"/> Prepare the NEBNext FFPE DNA Repair Mix and NEBNext End repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice.</p> <p>In a 0.2 ml thin-walled PCR tube, mix the following:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 27 µl Nuclease-free water <input type="checkbox"/> 20 µl Lambda DNA <input type="checkbox"/> 1 µl DNA CS (DCS) <input type="checkbox"/> 3.5 µl NEBNext FFPE DNA Repair Buffer <input type="checkbox"/> 2 µl NEBNext FFPE DNA Repair Mix <input type="checkbox"/> 3.5 µl Ultra II End-prep reaction buffer <input type="checkbox"/> 3 µl Ultra II End-prep enzyme mix <p><input type="checkbox"/> Mix gently by flicking the tube, and spin down.</p> <p><input type="checkbox"/> Using a thermal cycler, incubate at 20° C for 5 minutes and 65° C for 5 mins.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> AMPure XP bead clean-up</p>	
<ul style="list-style-type: none"> <input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing. <input type="checkbox"/> Transfer the DNA sample to a clean 1.5 ml Eppendorf DNA LoBind 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. <input type="checkbox"/> Repeat the previous step. <input type="checkbox"/> 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. <input type="checkbox"/> Remove the tube from the magnetic rack and resuspend the pellet in 61 µl Nuclease-free water. Incubate for 2 minutes at RT. <input type="checkbox"/> Pellet the beads on a magnet until the eluate is clear and colourless. <input type="checkbox"/> Remove and retain 61 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. 	
<p>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.</p>	

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<p>Adapter ligation and clean-up</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> Although the recommended 3rd party ligase is supplied with its own buffer, the ligation efficiency of Adapter Mix (AMX) is higher when using Ligation Buffer supplied within SQK-LSK109.</p>	
<p><input type="checkbox"/> Spin down Adapter Mix (AMX) and T4 Ligase from the NEBNext Quick Ligation Module (E6056), and place on ice.</p> <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Thaw the Elution Buffer (EB) at RT, mix by vortexing, spin down and place on ice.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> Depending on the wash buffer used in this section, the clean-up step after adapter ligation is designed to either enrich for long DNA fragments, or purify all fragments equally.</p>	
<p><input type="checkbox"/> To enrich for DNA fragments of 3 kb or longer, thaw one tube of L Fragment Buffer (LFB) at RT, mix by vortexing, spin down and place on ice.</p> <p><input type="checkbox"/> To retain DNA fragments shorter than 3 kb, thaw one tube of S Fragment Buffer (SFB) at RT, mix by vortexing, spin down and place on ice.</p> <p>In a 1.5 ml Eppendorf DNA LoBind tube, mix in the following order:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 60 µl DNA sample from the previous step <input type="checkbox"/> 25 µl Ligation Buffer (LNB) <input type="checkbox"/> 10 µl NEBNext Quick T4 DNA Ligase <input type="checkbox"/> 5 µl Adapter Mix (AMX) <p><input type="checkbox"/> Mix gently by flicking the tube, and spin down.</p> <p><input type="checkbox"/> Incubate the reaction for 10 minutes at RT.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> If you have omitted the AMPure purification step after DNA repair and end-prep, incubating the ligation reaction for longer than 10 minutes is not recommended.</p>	
<p><input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing.</p> <p><input type="checkbox"/> Add 40 µl of resuspended AMPure XP beads to the reaction and mix by pipetting.</p> <p><input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.</p> <p><input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.</p> <p><input type="checkbox"/> Wash the beads by adding either 250 µl L Fragment Buffer (LFB) or 250 µl S Fragment Buffer (SFB). 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.</p> <p><input type="checkbox"/> Repeat the previous step.</p>	

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<p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 15 µl Elution Buffer (EB). Incubate for 10 minutes at RT.</p> <p><input type="checkbox"/> Pellet the beads on a magnet until the eluate is clear and colourless.</p> <p>Remove and retain 15 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.</p> <p><input type="checkbox"/> Remove and retain the eluate which contains the DNA library in a clean 1.5 ml Eppendorf DNA LoBind tube</p> <p><input type="checkbox"/> Dispose of the pelleted beads</p>	
<p>The prepared library is used for loading into the flow cell. Store the library on ice until ready to load.</p>	
<p>Priming and loading the SpotON flow cell</p>	
<p><input type="checkbox"/> Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FLB) at RT before placing the tubes on ice as soon as thawing is complete.</p> <p><input type="checkbox"/> Mix the Sequencing Buffer (SQB) and Flush Buffer (FLB) tubes by vortexing, spin down and return to ice.</p> <p><input type="checkbox"/> Spin down the Flush Tether (FLT) tube, mix by pipetting, and return to ice.</p> <p><input type="checkbox"/> Open the lid of the nanopore sequencing device and slide the flow cell's priming port cover clockwise so that the priming port is visible.</p>	
<p>IMPORTANT</p> <p><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.</p>	
<p>After opening the priming port, check for small bubble under the cover. Draw back a small volume to remove any bubble (a few µls):</p> <p><input type="checkbox"/> Set a P1000 pipette to 200 µl</p> <p><input type="checkbox"/> Insert the tip into the priming port</p> <p><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</p> <p><input type="checkbox"/> 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 (FLB), and mix by pipetting up and down.</p> <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 LB tube by pipetting.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	

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<p>In a new tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 37.5 µl Sequencing Buffer (SQB) <input type="checkbox"/> 25.5 µl Loading Beads (LB), mixed immediately before use <input type="checkbox"/> 12 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"/> If your MinION was disconnected from the computer, plug it back in. <input type="checkbox"/> Choose the flow cell type from the selector box. Then mark the flow cell as "Selected". <input type="checkbox"/> Click the "New Experiment" button at the bottom left of the GUI. <p>On the New experiment popup screen, select the running parameters for your experiment from the individual tabs.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Output settings - FASTQ: The number of basecalls that MinKNOW will write in a single file. By default this is set to 4000 <input type="checkbox"/> Output settings - FAST5: The number of files that MinKNOW will write to a single folder. By default this is set to 4000 <input type="checkbox"/> Click "Begin Experiment". <p>Allow the script to run to completion.</p> <ul style="list-style-type: none"> <input type="checkbox"/> The MinKNOW Experiment page will indicate the progression of the script; this can be accessed through the "Experiment" tab that will appear at the top right of the screen <input type="checkbox"/> Monitor messages in the Message panel in the MinKNOW GUI <p>The basecalled read files are stored in :data\reads</p>	
<p>Progression of MinKNOW protocol script</p>	
<p>The running experiment screen</p> <p>Experiment summary information</p>	

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<p>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</p> <ul style="list-style-type: none"> <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"/> Stopping the experiment is achieved by clicking "Stop experiment" button at the top of the screen. <p><input type="checkbox"/> Check the temperature is approximately 34° C.</p> <p>Check pore occupancy in the channel panel at the top of the experimental view.</p> <ul style="list-style-type: none"> <input type="checkbox"/> A good library will be indicated by a higher proportion of light green channels in Sequencing than are in Pore. The combination of Sequencing and Pore indicates the number of active pores at any point in time. A low proportion of Sequencing channels will reduce the throughput of the run. <input type="checkbox"/> Recovering indicates channels that may become available for sequencing again. A high proportion of this may indicate additional clean up steps are required during your library preparation. <input type="checkbox"/> Inactive indicates channels that are no longer available for sequencing. A high proportion of these as soon as the run begins may indicate an osmotic imbalance. <input type="checkbox"/> Unclassified are channels that have not yet been assigned one of the above classifications <p><input type="checkbox"/> Monitor the pore occupancy</p> <p>Duty time plots</p> <p><input type="checkbox"/> Monitor the development of the read length histogram.</p> <p>Trace viewer</p>	
<p>Onward analysis of MinKNOW basecalled data</p>	
<ul style="list-style-type: none"> <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 FASTQ Control Experiment workflow. <p>Select the workflow parameters.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Select the quality score cut-off (this defaults to 7 unless changed) <input type="checkbox"/> Select whether the reads should be demultiplexed by barcode <input type="checkbox"/> If you are working with human data, please tick "Yes" in answer to "Is the data you are about to upload a whole or partial human genome?", and confirm that you have consent from the subject to upload the data. <ul style="list-style-type: none"> <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. <p>Click on VIEW REPORT.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Click on VIEW REPORT to navigate to the Metrichor 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 	

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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.	
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.	