

#1

A4 + G2



1D Native barcoding genomic DNA (with EXP-NBD103 and SQK-LSK109)

Version: NBE\_9065\_v109\_revA\_23May2018  
Last update: 09/08/2018

Flow Cell Number: FAJ182194 FLO-MINI06

DNA Samples: A4 + G2 HIGH # OF RECOVERING PORES IN RUN #1 SO RESTARTED

Before start checklist

Materials	Consumables	Equipment
<input type="checkbox"/> 1 µg (or 50-100 fmol) high molecular weight genomic DNA for every sample to be barcoded	<input checked="" type="checkbox"/> Agencourt AMPure XP beads	<input type="checkbox"/> Hula mixer (gentle rotator mixer)
<input type="checkbox"/> Native Barcoding Expansion 1-12 (EXP-NBD103)	<input type="checkbox"/> NEBNext FFPE Repair Mix (M6630)	<input type="checkbox"/> Magnetic separator, suitable for 1.5 ml Eppendorf tubes
<input type="checkbox"/> Ligation Sequencing Kit (SQK-LSK109)	<input type="checkbox"/> NEBNext End repair / dA-tailing Module (E7546)	<input type="checkbox"/> Microfuge
<input type="checkbox"/> Flow Cell Priming Kit (EXP-FLP001)	<input type="checkbox"/> NEB Blunt/TA Ligase Master Mix (M0367)	<input type="checkbox"/> Vortex mixer
	<input type="checkbox"/> NEBNext Quick Ligation Module (E6056)	<input type="checkbox"/> Thermal cycler
	<input type="checkbox"/> 1.5 ml Eppendorf DNA LoBind tubes	<input type="checkbox"/> Ice bucket with ice
	<input type="checkbox"/> 0.2 ml thin-walled PCR tubes	<input type="checkbox"/> Timer
	<input type="checkbox"/> Nuclease-free water (e.g. ThermoFisher, cat # AM9937)	<input type="checkbox"/> Pipettes and pipette tips P2, P10, P20, P100, P200, P1000
	<input type="checkbox"/> Freshly prepared 70% ethanol in nuclease-free water	

INSTRUCTIONS	NOTES/OBSERVATIONS
<p>Preparing input DNA</p> <p><input type="checkbox"/> Record the quality, quantity and size of the DNA.</p> <p>IMPORTANT</p> <p>Criteria for input DNA</p> <p><input type="checkbox"/> Purity as measured using Nanodrop - OD 260/280 of 1.8 and OD 260/230 of 2.0-2.2</p> <p><input type="checkbox"/> Average fragment size, as measured by pulse-field, or low percentage agarose gel analysis &gt;30 kb</p> <p><input type="checkbox"/> Input mass, as measured by Qubit - 1 µg, or 100-200 fmol for short-fragment libraries</p> <p><input type="checkbox"/> No detergents or surfactants in the buffer</p> <p>Prepare the DNA in Nuclease-free water.</p> <p><input type="checkbox"/> Transfer 1 µg genomic DNA into a DNA LoBind tube</p> <p><input type="checkbox"/> Adjust the volume to 48 µl with Nuclease-free water</p> <p><input type="checkbox"/> Mix thoroughly by inversion avoiding unwanted shearing</p> <p><input type="checkbox"/> Spin down briefly in a microfuge</p>	<p>RUN #1</p> <p>A4 - 30ul + 18ul H<sub>2</sub>O</p> <p><del>A4 - 28ul + 20ul H<sub>2</sub>O</del></p> <p>G2 - 35ul + 13ul H<sub>2</sub>O</p> <p>RUN #2</p> <p>A4 - 95.6 ng/ul - 10.5ul + 37.5 H<sub>2</sub>O</p> <p>G2 - 42.4 ng/ul - 23.5ul + 24.5 H<sub>2</sub>O</p>

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DNA Samples: .....

INSTRUCTIONS	NOTES/OBSERVATIONS
<p><b>Check your flow cell</b></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> <ul style="list-style-type: none"> <li><input type="checkbox"/> If running a MinION on the same host computer, plug the MinION into the computer.</li> <li><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.</li> <li><input type="checkbox"/> Choose the flow cell type from the selector box. Then mark the flow cell as "Selected":</li> </ul> <p>Click "Check flow cells" at the bottom of the screen.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> R9.4.1 FLO-MIN106</li> <li><input type="checkbox"/> R9.5.1 FLO-MIN107</li> </ul> <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>	
<p>Flow cell check complete.</p>	
<p><b>DNA fragmentation (optional, for lower inputs)</b></p> <p>OPTIONAL</p> <p>Covaris g-TUBE</p> <p><input type="checkbox"/> Transfer the genomic DNA sample in 49 µl to the Covaris g-TUBE.</p> <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"> <li><input type="checkbox"/> Spin the g-TUBE for 1 minute</li> <li><input type="checkbox"/> Remove and check all the DNA has passed through the g-TUBE</li> <li><input type="checkbox"/> If DNA remains in the upper chamber, spin again for 1 minute at the same speed</li> </ul> <p>Invert the g-TUBE and spin again for 1 minute to collect the fragmented DNA.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Remove g-TUBE, invert the tube and replace into the centrifuge</li> <li><input type="checkbox"/> Spin the g-TUBE for 1 minute</li> <li><input type="checkbox"/> Remove and check the DNA has passed into the lower chamber</li> <li><input type="checkbox"/> If DNA remains in the upper chamber, spin again for 1 minute</li> <li><input type="checkbox"/> Remove g-TUBE</li> </ul> <p><input type="checkbox"/> Transfer the 49 µl fragmented DNA to a clean 1.5 ml Eppendorf DNA LoBind tube.</p>	
<p>Analyse 1 µl of the fragmented DNA for fragment size, quantity and quality.</p>	
<p>48 µl of fragmented DNA is taken into the next step.</p>	

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INSTRUCTIONS	NOTES/OBSERVATIONS
<p><b>DNA repair and end-prep</b></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"> <li><input type="checkbox"/> 48 µl DNA</li> <li><input type="checkbox"/> 3.5 µl NEBNext FFPE DNA Repair Buffer</li> <li><input type="checkbox"/> 2 µl NEBNext FFPE DNA Repair Mix</li> <li><input type="checkbox"/> 3.5 µl Ultra II End-prep reaction buffer</li> <li><input type="checkbox"/> 3 µl Ultra II End-prep enzyme mix</li> </ul> <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><i>IT IS * POSSIBLE * THAT THE CAPS WERE SWITCHED AND THAT THE SAMPLE IDS WERE SWAPPED - CHECK FOR E-MARKING IN SAMPLES</i></p> <p><i>→ HOWEVER, POST REPAIR CONCENTRATIONS SUGGEST THAT THE NUMBERS ARE CORRECT</i></p>
<p><b>IMPORTANT</b></p> <p><input type="checkbox"/> AMPure XP bead clean-up</p>	
<ul style="list-style-type: none"> <li><input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing.</li> <li><input type="checkbox"/> Transfer the DNA sample to a clean 1.5 ml Eppendorf DNA LoBind tube.</li> <li><input type="checkbox"/> Add 60 µl of resuspended AMPure XP beads to the end-prep reaction and mix by pipetting.</li> <li><input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.</li> <li><input type="checkbox"/> Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.</li> <li><input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.</li> <li><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.</li> <li><input type="checkbox"/> Repeat the previous step.</li> <li><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.</li> <li><input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 25 µl Nuclease-free water. Incubate for 2 minutes at RT.</li> <li><input type="checkbox"/> Pellet the beads on a magnet until the eluate is clear and colourless.</li> <li><input type="checkbox"/> Remove and retain 25 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.</li> <li><input type="checkbox"/> Quantify 1 µl of end-prepped DNA using a Qubit fluorometer - recovery aim &gt; 700 ng.</li> </ul>	<p><i>A4 = 104 ng/µL</i></p> <p><i>G2 = 55 ng/µL</i></p>
<p>Take forward the repaired and end-prepped DNA into the native barcode ligation step.</p>	

*RUN # 2*

*A4 = 33.6 ng/µL = 840 ng*

*G2 = 29.6 ng/µL = 715 ng*

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DNA Samples: .....

STOP HERE  
HERE  
(SAMPLES  
DILUTED  
"LIBRARY")

INSTRUCTIONS	NOTES/OBSERVATIONS
<p><b>Native barcode ligation</b></p> <p><input type="checkbox"/> Thaw the Native Barcodes at RT, enough for one barcode per sample. Mix the barcodes by pipetting, and place them on ice.</p> <p><input type="checkbox"/> Select a unique barcode for every sample to be run together on the same flow cell, from the provided 12 barcodes. Up to 12 samples can be barcoded and combined in one experiment.</p> <p><input type="checkbox"/> Dilute 500 ng of each end-prepped sample to be barcoded to 22.5 µl in Nuclease-free water.</p> <p>Add the reagents in the order given below, mixing by flicking the tube between each sequential addition:</p> <p><input type="checkbox"/> 22.5 µl 500 ng end-prepped DNA</p> <p><input type="checkbox"/> 2.5 µl Native Barcode</p> <p><input type="checkbox"/> 25 µl Blunt/TA Ligase Master Mix</p> <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><input type="checkbox"/> Prepare the AMPure XP beads for use; resuspend by vortexing.</p> <p><input type="checkbox"/> Add 50 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"/> Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.</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"/> 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.</p> <p><input type="checkbox"/> Repeat the previous step.</p> <p><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.</p> <p><input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 26 µl Nuclease-free water. Incubate for 2 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 26 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>A4 = 5 µl + 17.5 µl H<sub>2</sub>O</p> <p>G2 = 10 µl + 12.5 µl H<sub>2</sub>O</p> <p>RUN #2</p> <p>A4 = 33.6 ng/µl → 15 µl + 7.5 µl H<sub>2</sub>O</p> <p>G2 = 28.6 ng/µl → 17.5 µl + 5 µl H<sub>2</sub>O</p> <p>NB01 - A4 } SAME FOR</p> <p>NB02 - G2 } RUN #2</p> <p>A4 = 29.8 ng/µl</p> <p>G2 = 24.0 ng/µl</p> <p>RUN #2</p>
<p>Quantify 1 µl of eluted sample using a Qubit fluorometer.</p>	<p>A4 = 11.3 ng/µl</p> <p>G2 = 9.68 ng/µl</p>
<p><input type="checkbox"/> Pool equimolar amounts of each barcoded sample into a DNA LoBind 1.5 ml Eppendorf tube, ensuring that sufficient sample is combined to produce a pooled sample of 700 ng total.</p>	

A4 - 350 ng = 12 µl

G2 - 350 ng = 15 µl

RUN #2

7 + 200 =

1400 - 7

= 1393

A4 - 350 ng =

G2 - 350 ng =

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<p><input type="checkbox"/> Quantify 1 µl of pooled and barcoded DNA using a Qubit fluorometer. <i>25 ng/µl × 27 µl =</i></p> <p><input type="checkbox"/> Dilute 700 ng pooled sample to 50 µl in Nuclease-free water. <i>26 µl + 2 = 28 µl + 22 µl H<sub>2</sub>O</i></p>	
<p><b>Adapter ligation and clean-up</b></p>	
<p><b>IMPORTANT</b></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"/> Thaw one tube of Barcode Adapter Mix (BAM 1D) at RT, spin down, mix by pipetting and place on ice.</p> <p><input type="checkbox"/> Thaw Elution Buffer (EB) and NEBNext Quick Ligation Reaction Buffer (5x) at RT, mix by vortexing, spin down and place on ice. Check the contents of each tube are clear of any precipitate.</p> <p><input type="checkbox"/> Spin down the T4 Ligase, and place on ice.</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>Taking the pooled and barcoded DNA, perform adapter ligation as follows, mixing by flicking the tube between each sequential addition.</p> <p><input type="checkbox"/> 50 µl 700 ng pooled barcoded sample</p> <p><input type="checkbox"/> 20 µl Barcode Adapter Mix (BAM 1D)</p> <p><input type="checkbox"/> 20 µl NEBNext Quick Ligation Reaction Buffer (5X)</p> <p><input type="checkbox"/> 10 µl Quick T4 DNA Ligase</p> <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><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 adapter ligation reaction from the previous step 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"/> Place on magnetic rack, allow beads to pellet and pipette off 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> <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>	

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INSTRUCTIONS	NOTES/OBSERVATIONS
<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><input type="checkbox"/> Quantify 1 µl of adapter ligated DNA using a Qubit fluorometer - recovery aim ~430 ng.</p>	<p><math>36.8 \text{ ng/}\mu\text{l} \times 15 \text{ }\mu\text{l} = 552 \text{ ng}</math></p>
<p>The prepared library is used for loading into the MinION flow cell. Store the library on ice until ready to load.</p>	<p><i>PUR#2</i> <math>37.0 \text{ ng/}\mu\text{l} \times 14 \text{ }\mu\text{l} = 518 \text{ ng}</math></p>
<b>Priming and loading the SpotON flow cell</b>	
<p><b>IMPORTANT</b></p> <p><input type="checkbox"/> Please note that the Sequencing Tether (SQT) tube will NOT be used in this protocol. It is provided in the kit for potential future product compatibility.</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><b>IMPORTANT</b></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><b>IMPORTANT</b></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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Flow Cell Number: .....

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<p>In a new tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> 37.5 µl Sequencing Buffer (SQB)</li> <li><input type="checkbox"/> 25.5 µl Loading Beads (LB), mixed immediately before use</li> <li><input type="checkbox"/> 12 µl DNA library</li> </ul> <p>Complete the flow cell priming:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Gently lift the SpotON sample port cover to make the SpotON sample port accessible.</li> <li><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.</li> <li><input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading.</li> <li><input type="checkbox"/> Add 50 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.</li> <li><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.</li> </ul>	
<p><b>Starting a sequencing run</b></p>	
<ul style="list-style-type: none"> <li><input type="checkbox"/> Double-click the MinkNOW icon located on the desktop to open the MinkNOW GUI.</li> <li><input type="checkbox"/> If your MinION was disconnected from the computer, plug it back in.</li> <li><input type="checkbox"/> Choose the flow cell type from the selector box. Then mark the flow cell as "Selected".</li> <li><input type="checkbox"/> Click the "New Experiment" button at the bottom left of the GUI.</li> </ul> <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"> <li><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</li> <li><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</li> <li><input type="checkbox"/> Click "Begin Experiment".</li> </ul> <p>Allow the script to run to completion.</p> <ul style="list-style-type: none"> <li><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</li> <li><input type="checkbox"/> Monitor messages in the Message panel in the MinkNOW GUI</li> </ul> <p>The basecalled read files are stored in :data\reads</p>	
<p><b>Progression of MinkNOW protocol script</b></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 Flow Cell Check</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> If there is a significant reduction in the numbers, restart MinKNOW.</li> <li><input type="checkbox"/> If the numbers are still significantly different, close down the host computer and reboot.</li> <li><input type="checkbox"/> When the numbers are similar to those reported at the end of the Flow Cell Check, restart the experiment on the Connection page. There is no need to load any additional library after restart.</li> <li><input type="checkbox"/> Stopping the experiment is achieved by clicking "Stop experiment" button at the top of the screen.</li> </ul> <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"> <li><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.</li> <li><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.</li> <li><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.</li> <li><input type="checkbox"/> Unclassified are channels that have not yet been assigned one of the above classifications</li> </ul> <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><b>Onward analysis of MinKNOW basecalled data</b></p>	
<ul style="list-style-type: none"> <li><input type="checkbox"/> Open the Desktop Agent using the desktop shortcut.</li> <li><input type="checkbox"/> Click on the New Workflow tab in the Desktop Agent and select the FASTQ barcoding workflow.</li> </ul> <p>Select the workflow parameters.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Select the quality score cut-off (this defaults to 7 unless changed)</li> <li><input type="checkbox"/> Select "Yes" in answer to "Detect barcode?"</li> <li><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.</li> </ul> <ul style="list-style-type: none"> <li><input type="checkbox"/> Check the correct settings are selected in the Desktop Agent.</li> <li><input type="checkbox"/> Click "Start Run" to start data analysis.</li> <li><input type="checkbox"/> Follow the progression of upload and download of read files in the Desktop Agent.</li> </ul>	



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Click on VIEW REPORT. <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	
<b>Close down MinKNOW and the Desktop Agent</b>	
<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.	
<b>Prepare the flow cell for re-use or return to Oxford Nanopore.</b>	
<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.	

8/25/18

Zymo ONE STEP INHIBITOR REMOVAL

COMBINED ALL DNA SOURCES FROM DNA PREP #2 (COLUMN TISSUE)

RAN THROUGH Zymo KIT

QUBIT HS RESULTS (1  $\mu$ l SAMPLE) TOTAL VOL 55-70  $\mu$ l

A4 95.6 ng/ $\mu$ l

G2 42.4 ng/ $\mu$ l

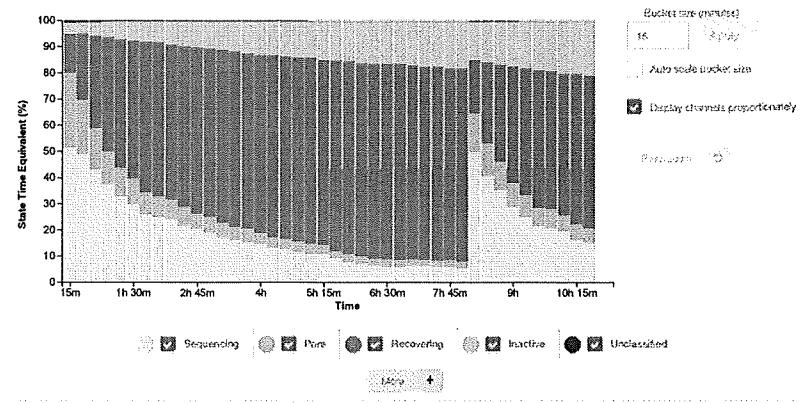
$$11 \times 200 = 2200 - 11 = 2189$$

+ 11 REAGENT

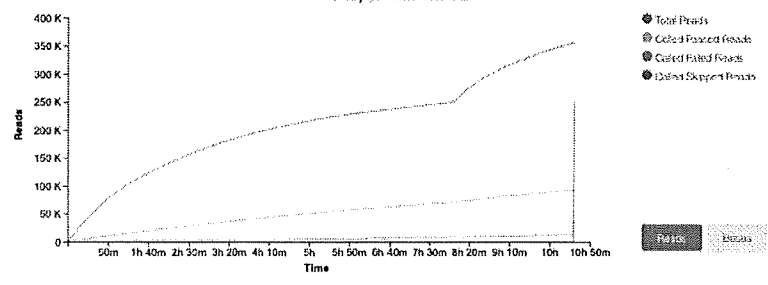
RUN #1

**Experiment complete**  
[Export PDF Report](#)

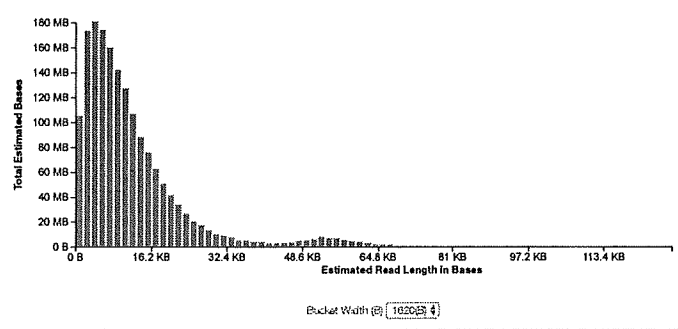
**Duty Time**  
 Summary of channel states over time



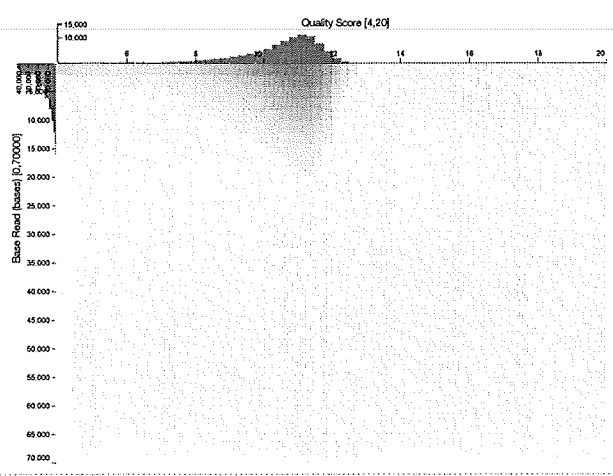
**Cumulative Throughput**  
 Throughput of seq over time



**Read Length Histogram**  
 Summary read length distribution



**Basecall**  
 Histogram of read length vs basecall quality score



- Messages**
- advancing to group 2
  - Starting Sequencing
  - Reached target temperature
  - waiting for temperature to be within acceptable bounds
  - Experimental Parameters Complete
  - Setting Experimental Parameters
  - calibration finished successfully
  - Starting Calibration
  - Experimental Parameters Complete
  - Finished Mux Scan
  - Group 3 has 334 active channels
  - Group 4 has 131 active channels
  - Group 2 has 448 active channels
  - Group 1 has 496 active channels
  - a total of 1469 wells have been selected and split into the following groups
  - Processing Mux 4
  - advancing to group 4
  - Processing Mux 4
  - advancing to group 3
  - Processing Mux 3
  - advancing to group 2
  - Processing Mux 2
  - advancing to group 2
  - Processing Mux 1
  - Starting Mux Scan
  - Reached target temperature
  - waiting for temperature to be within acceptable bounds
  - Experimental Parameters Complete
  - Setting Experimental Parameters
  - calibration finished successfully
  - Starting Calibration
  - Experimental Parameters Complete
  - Finished Platform QC
  - group 4 has 146 active single pores
  - group 3 has 343 active single pores
  - group 2 has 451 active single pores
  - group 1 has 496 active single pores
  - A total of 1433 single pores were detected, these have been split into 4 groups as follows
  - processing mux 4



RUN # 2 SAME FLOW CELL, NEW LIBRARY

Experiment group: M\_02\_PXA\_rn2 Sample ID: A4\_G2\_55A\_rn2 Flow cell ID: F41845A Flow cell product code: FLO-MIN10 Kit ID: 506-18C102 Current output directory: /output/1000

Messages  
 1/26

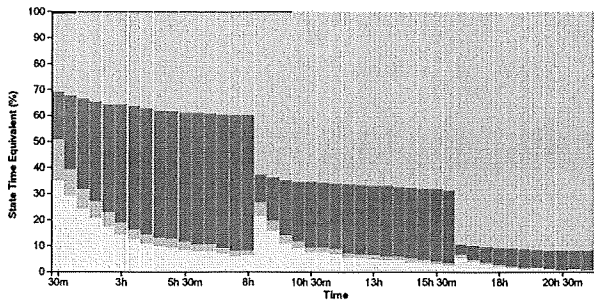
### Finishing up...

This run is taking a while if your experiment had local basecalling enabled

Continue | Export PDF Report

### Duty Time

Summary of channel states over time

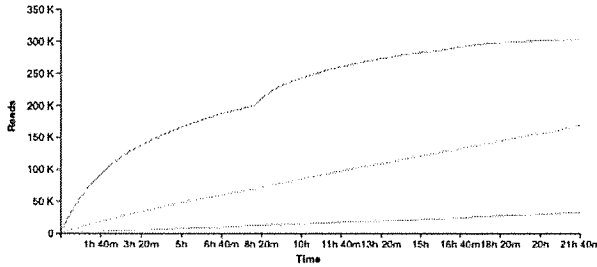


Bucket size (minutes): 30  
 Auto scale bucket sizes  
 Display channels proportionately  
 Export names

Sequencing Pore Recovering Inactive Unclassified

### Cumulative Throughput

Throughput of run over time

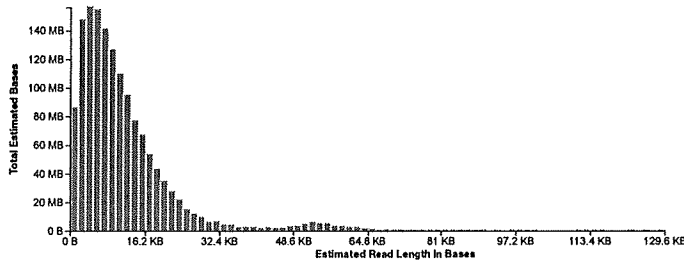


Total Reads  
 Called Poreseq Reads  
 Called Fluidigm Reads  
 Called Skipped Reads

Reads | Stats

### Read Length Histogram

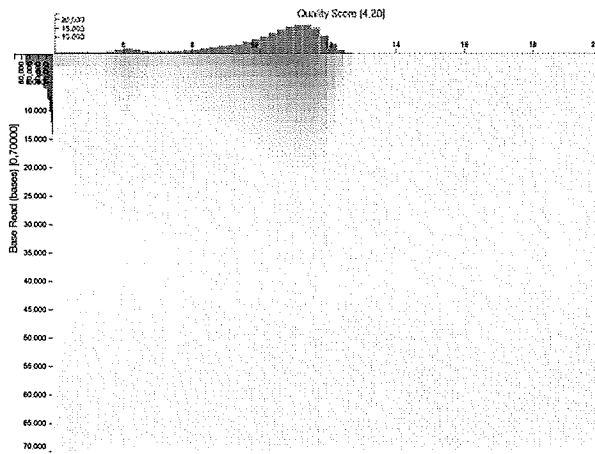
Summary read length distribution



Bucket Width (B): 16200

### Basecall

Histogram of read length vs basecall quality score



- 1 advancing to group 3  
M22564 21 hours ago
- 1 advancing to group 2  
M22564 21 hours ago
- 1 Starting Sequencing  
M22564 21 hours ago
- 1 Reached target temperature  
M22564 21 hours ago
- 1 waiting for temperature to be within acceptable bounds  
M22564 21 hours ago
- 1 Experimental Parameters Complete  
M22564 21 hours ago
- 1 Setting Experimental Parameters  
M22564 21 hours ago
- 1 calibration finished successfully  
M22564 21 hours ago
- 1 Starting Calibration  
M22564 21 hours ago
- 1 Experimental Parameters Complete  
M22564 21 hours ago
- 1 Finished Mux Scan  
M22564 21 hours ago
- 1 Group 4 has 4 active channels  
M22564 21 hours ago
- 1 Group 3 has 56 active channels  
M22564 21 hours ago
- 1 Group 2 has 199 active channels  
M22564 21 hours ago
- 1 Group 1 has 373 active channels  
M22564 21 hours ago
- 1 A total of 632 wells have been selected and split into the following groups  
M22564 21 hours ago
- 1 Processing Mux 4  
M22564 21 hours ago
- 1 advancing to group 4  
M22564 21 hours ago
- 1 Processing Mux 3  
M22564 21 hours ago
- 1 advancing to group 3  
M22564 21 hours ago
- 1 Processing Mux 2  
M22564 21 hours ago
- 1 advancing to group 2  
M22564 21 hours ago
- 1 Processing Mux 1  
M22564 21 hours ago
- 1 Starting Mux Scan  
M22564 21 hours ago
- 1 Reached target temperature  
M22564 21 hours ago
- 1 waiting for temperature to be within acceptable bounds  
M22564 21 hours ago
- 1 Experimental Parameters Complete  
M22564 21 hours ago
- 1 Setting Experimental Parameters  
M22564 21 hours ago
- 1 calibration finished successfully  
M22564 21 hours ago
- 1 Starting Calibration  
M22564 21 hours ago
- 1 Experimental Parameters Complete  
M22564 21 hours ago
- 1 group 4 has 20 active single pores  
M22564 1 hour ago
- 1 Finished Platform QC  
M22564 1 hour ago
- 1 group 3 has 106 active single pores  
M22564 1 hour ago
- 1 group 2 has 281 active single pores  
M22564 1 hour ago
- 1 A total of 828 single pores were detected, these have been split into 4 groups as follows  
M22564 1 hour ago
- 1 group 1 has 421 active single pores  
M22564 1 hour ago

