

Ligation Sequencing gDNA V14 - human sample (N50 30 kb) on PromethION (SQK-LSK114)

Version: GDH_9174_v114_revN_10Nov2022
Last update: 29/02/2024



Flow Cell Number:

DNA Samples:

Before start checklist

Materials

☐ 10 µg of high molecular weight genomic DNA or 5 x 10⁶ cells (e.g. cell culture or tissue)

☐ Short Fragment Eliminator (EXP-SFE001) kit

☐ Ligation Sequencing Kit V14 (SQK-LSK114)

☐ Flow Cell Wash Kit (EXP-WSH004)

☐ Sequencing Auxiliary Vials V14 (EXP-AUX003)

Consumables

☐ NEBNext® Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (cat # E7180S or E7180L). Alternatively, you can use the NEBNext® products below:

☐ NEBNext FFPE Repair Mix (NEB, M6630)

☐ NEBNext® Ultra™ II End Repair/dA-Tailing Module (E7546)

☐ NEBNext Quick Ligation Module (NEB, E6056)

☐ Puregene Cell Kit (QIAGEN, Cat. # 158043)

☐ Nuclease-free water (e.g. ThermoFisher, cat # AM9937)

☐ 15 ml Falcon tubes

☐ 2 ml Eppendorf DNA LoBind tubes

☐ 1.5 ml Eppendorf DNA LoBind tubes

☐ 0.2 ml thin-walled PCR tubes

☐ Freshly prepared 70% ethanol in nuclease-free water

☐ Phosphate Buffered Saline (PBS), pH 7.4 (Thermo Fisher, 10010023)

☐ Isopropanol, 100% (Fisher, 10723124)

☐ TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) (Fisher scientific, 10224683)

☐ Inoculation loop or disposable tweezers

☐ (Optional) TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

☐ Qubit™ Assay Tubes (Invitrogen, Q32856)

☐ Qubit dsDNA BR Assay Kit (Invitrogen, Q32850)

☐ Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)

☐ Agilent Genomic DNA 165 kb Analysis Kit

Equipment

☐ Hula mixer (gentle rotator mixer)

☐ Magnetic rack, suitable for 1.5 ml Eppendorf tubes

☐ Microfuge

☐ Vortex mixer

☐ Thermal cycler

☐ Centrifuge and rotor suitable for 15 ml Falcon tubes

☐ Incubator or water bath set at 37°C and 50°C

☐ P2 pipette and tips

☐ Ice bucket with ice

☐ Timer

☐ Heating block

☐ Ice bucket with ice

☐ Agilent Femto Pulse System (or equivalent for read length QC)

☐ Qubit fluorometer (or equivalent for QC check)

☐ Pipettes and pipette tips P10, P20, P100, P200, P1000

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<p>Human cell line DNA extraction using the QIAGEN Puregene Cell Kit</p> <p>IMPORTANT</p> <p><input type="checkbox"/> We recommend using wide-bore pipette tips during sample and library preparation for N50s >20 kb to avoid mechanical shearing of your sample.</p> <p><input type="checkbox"/> Prepare a 1.5 ml Eppendorf DNA LoBind tube with approximately 1 ml of 70% ethanol and store on ice to cool.</p> <p><input type="checkbox"/> We recommend to harvest and pellet 5×10^6 cells in a 1.5 ml Eppendorf DNA LoBind tube. However, this may vary depending on your sample type. If any liquid remains associated with the pellet, spin down the cells again, then aspirate and discard the remaining supernatant.</p> <p><input type="checkbox"/> Add 200 μl of 1x PBS to pelleted cells.</p> <p><input type="checkbox"/> Centrifuge at 300 x g for 3 minutes.</p> <p><input type="checkbox"/> Without disturbing the pellet, aspirate and discard the supernatant.</p> <p><input type="checkbox"/> Add 2 ml of Cell Lysis Solution to the washed cell pellet and resuspend the cells using a wide-bore pipette tip.</p> <p><input type="checkbox"/> Transfer the resuspended cells to a 15 ml Falcon. If clumps of cells remain, gently invert the tube to ensure resuspension.</p> <p><input type="checkbox"/> Incubate the sample at 37°C for 30 minutes.</p> <p><input type="checkbox"/> Add 700 μl of the Protein Precipitation Solution to the lysed cells and mix by vortexing for three pulses of 5 seconds.</p> <p><input type="checkbox"/> Centrifuge the sample at 2000 x g for 5 minutes.</p> <p><input type="checkbox"/> Transfer the supernatant to a new 15 ml falcon tube and add 2.5 ml isopropanol at RT. Discard the pellet.</p> <p><input type="checkbox"/> Mix by gently inverting the tube 50 times.</p> <p><input type="checkbox"/> Spool the DNA using an inoculation loop or disposable tweezers.</p> <p><input type="checkbox"/> Briefly dip the spooled DNA in the 1.5 ml Eppendorf DNA LoBind tube containing cold 70% ethanol and allow to air dry for a few seconds.</p> <p><input type="checkbox"/> Transfer the inoculation loop or tweezers with the spooled DNA to a 1.5 ml Eppendorf DNA LoBind tube containing 250 μl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and allow the DNA to gently dislodge from the loop/tweezers.</p> <p><input type="checkbox"/> Incubate the DNA pellet for 2 hours at 50°C, occasionally mixing the tube by gentle inversion to aid dissolving the pellet. Alternatively, the DNA pellet can be left overnight at RT.</p> <p><input type="checkbox"/> Quantify the sample three times using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to the next step.</p> <p>Take the sample in 250 μl TE buffer forwards into the next step or the sample can be stored at 4°C overnight.</p>	

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INSTRUCTIONS	NOTES/OBSERVATIONS
<p>Size select for 30 kb input using Short Fragment Eliminator kit</p> <ul style="list-style-type: none"> <input type="checkbox"/> In a 1.5 ml Eppendorf DNA LoBind tube, prepare 10 µg DNA in 100 µl of Nuclease-free water to a final concentration of ~100 ng/µl. <input type="checkbox"/> Add an equal volume of the Short Fragment Eliminator (SFE) buffer to the DNA sample and mix thoroughly by gently flicking the tube until homogenous. <input type="checkbox"/> Place the tube in the centrifuge and note the orientation of the tube within the rotor. <input type="checkbox"/> Centrifuge the sample at 10,000 x g at RT for 30 minutes. <input type="checkbox"/> Aspirate and discard the supernatant, taking care not to disturb the pellet. <input type="checkbox"/> Without disturbing the pellet, add 200 µl of freshly prepared 70% ethanol to the tube. Centrifuge the sample at 10,000 x g for 3 minutes at the same orientation used in for the previous centrifuge step. Pipette off the ethanol and discard. <input type="checkbox"/> Repeat the previous step. <input type="checkbox"/> Add 50 µl of Nuclease-free water to the DNA pellet and mix by gently flicking the tube. <input type="checkbox"/> Incubate the tube in a heat block at 37°C for 30 minutes. Gently agitate the solution by flicking every 5 minutes to aid with resuspension. Alternatively, use an incubated shaking heat block at 37°C, 300 rpm for 30 minutes. <input type="checkbox"/> Gently mix the tube contents by pipetting up and down using a wide-bore tip. <input type="checkbox"/> Quantify the sample three times using the Qubit dsDNA BR Assay Kit, ensuring that replicate Qubit measurements are consistent before continuing to library preparation. 	
Quantify sample read length using the Agilent Femto Pulse System. An N50 of 30 kb or above is to be expected.	
Take forwards 3 µg of sample into the library preparation step or the sample can be stored at 4°C overnight.	
DNA repair and end-prep	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> We recommend using wide-bore pipette tips during sample and library preparation for N50s >20 kb to avoid mechanical shearing of your sample. 	
<input type="checkbox"/> Thaw DNA Control Sample (DCS) at RT, spin down, mix by pipetting, and place on ice.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
<p>Prepare the NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Thaw all reagents on ice. <input type="checkbox"/> Flick and/or invert the reagent tubes to ensure they are well mixed. Note: Do not vortex the FFPE DNA Repair Mix or Ultra II End Prep Enzyme Mix. <input type="checkbox"/> Always spin down tubes before opening for the first time each day. <input type="checkbox"/> The Ultra II End Prep Buffer and FFPE DNA Repair Buffer may have a little precipitate. Allow the mixture to come to RT and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for 30 seconds to solubilise any precipitate. Note: It is important the buffers are mixed well by vortexing. <input type="checkbox"/> The FFPE DNA Repair Buffer may have a yellow tinge and is fine to use if yellow. <p>Prepare the extracted gDNA in Nuclease-free water:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Transfer 3 µg of gDNA into a 1.5 ml Eppendorf DNA LoBind tube <input type="checkbox"/> Adjust the volume to 47 µl with Nuclease-free water <input type="checkbox"/> Mix thoroughly by pipetting up and down, or by flicking the tube <input type="checkbox"/> Spin down briefly in a microfuge <p>In a 0.2 ml thin-walled PCR tube, mix the following:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 47 µl gDNA from the previous step <input type="checkbox"/> 1 µl DNA CS <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 <ul style="list-style-type: none"> <input type="checkbox"/> Thoroughly mix the reaction by gently pipetting and briefly spinning down. <input type="checkbox"/> Using a thermal cycler, incubate at 20°C for 5 minutes and 65°C for 5 minutes. <input type="checkbox"/> Resuspend the AMPure XP Beads (AXP) 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 the AMPure XP Beads (AXP) to the end-prep reaction and mix 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 for 10 minutes until the supernatant is clear and colourless. Keep the tube on the magnet, and pipette off the supernatant. <input type="checkbox"/> 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. <input type="checkbox"/> Repeat the previous step. 	

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<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, for at least 1 minute.	
<input type="checkbox"/> Remove and retain 61 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.	
Quantify 1 µl of eluted sample using a Qubit fluorometer.	
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.	
Adapter ligation and clean-up	
IMPORTANT <input type="checkbox"/> Although the recommended third-party ligase is supplied with its own buffer, the ligation efficiency of the Ligation Adapter (LA) is higher when using the Ligation Buffer (LNB) supplied in the Ligation Sequencing Kit.	
<input type="checkbox"/> Spin down the Ligation Adapter (LA) and Quick T4 Ligase, and place on ice.	
<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.	
<input type="checkbox"/> Thaw the Elution Buffer (EB) at RT and mix by vortexing. Then spin down and place on ice.	
<input type="checkbox"/> Thaw the Long Fragment Buffer (LFB) 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:	
<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 Ligation Adapter (LA)	
<input type="checkbox"/> Thoroughly mix the reaction by gently pipetting and briefly spinning down.	
<input type="checkbox"/> Incubate the reaction for 10 minutes at RT.	
<input type="checkbox"/> Resuspend the AMPure XP Beads (AXP) by vortexing.	
<input type="checkbox"/> Add 40 µl of resuspended AMPure XP Beads (AXP) to the reaction and mix by flicking the tube.	
<input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.	
<input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant when clear and colourless.	

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<input type="checkbox"/> Wash the beads by adding 250 µl Long Fragment Buffer (LFB). Flick the beads to resuspend, spin down, then return the tube to the magnetic rack and allow the beads to pellet for at least 5 minutes. Remove the supernatant 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 supernatant. 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 25 µl Elution Buffer (EB). Spin down and incubate for 10 minutes at 37°C. <input type="checkbox"/> Pellet the beads on a magnet for 10 minutes until the eluate is clear and colourless. <input type="checkbox"/> Remove and retain 25 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.	
<input type="checkbox"/> Split your library into three libraries of 300 ng (10-20 fmol) in 32 µl using Elution Buffer (EB).	
IMPORTANT <input type="checkbox"/> For libraries with an N50 of 30 kb, we recommend loading 300 ng (10-20 fmol) of your final library onto the R10.4.1 flow cell.	
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 PromethION Flow Cell	
IMPORTANT <input type="checkbox"/> This kit is only compatible with R10.4.1 flow cells (FLO-PRO114M).	
<input type="checkbox"/> Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT before mixing by vortexing. Then spin down and store on ice. To prepare the flow cell priming mix, combine Flow Cell Tether (FCT) and Flow Cell Flush (FCF), as directed below. Mix by vortexing at RT. <input type="checkbox"/> 1,170 µl Flow Cell Flush (FCF) <input type="checkbox"/> 30 µl Flow Cell Tether (FCT)	
IMPORTANT <input type="checkbox"/> After taking flow cells out of the fridge, wait 20 minutes before inserting the flow cell into the PromethION for the flow cell to come to RT. Condensation can form on the flow cell in humid environments. Inspect the gold connector pins on the top and underside of the flow cell for condensation and wipe off with a lint-free wipe if any is observed. Ensure the heat pad (black pad) is present on the underside of the flow cell.	
For PromethION 2 Solo, load the flow cell(s) as follows: <input type="checkbox"/> Place the flow cell flat on the metal plate. <input type="checkbox"/> Slide the flow cell into the docking port until the gold pins or green board cannot be seen.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
<p>For the PromethION 24/48, load the flow cell(s) into the docking ports:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Line up the flow cell with the connector horizontally and vertically before smoothly inserting into position. <input type="checkbox"/> Press down firmly onto the flow cell and ensure the latch engages and clicks into place. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> Insertion of the flow cells at the wrong angle can cause damage to the pins on the PromethION and affect your sequencing results. If you find the pins on a PromethION position are damaged, please contact support@nanoporetech.com for assistance. 	
<ul style="list-style-type: none"> <input type="checkbox"/> Slide the inlet port cover clockwise to open. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> 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. 	
<p>After opening the inlet port, draw back a small volume to remove any air bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette tip to 200 µl. <input type="checkbox"/> Insert the tip into the inlet port. <input type="checkbox"/> Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip. <ul style="list-style-type: none"> <input type="checkbox"/> Load 500 µl of the priming mix into the flow cell via the inlet port, avoiding the introduction of air bubbles. Wait five minutes. During this time, prepare the library for loading using the next steps in the protocol. <input type="checkbox"/> Thoroughly mix the contents of the Library Beads (LIB) by pipetting. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use. 	
<p>In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 100 µl Sequencing Buffer (SB) <input type="checkbox"/> 68 µl Library Beads (LIB) thoroughly mixed before use, or Library Solution (LIS) <input type="checkbox"/> 32 µl DNA library <ul style="list-style-type: none"> <input type="checkbox"/> Complete the flow cell priming by slowly loading 500 µl of the priming mix into the inlet port. <input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading. <input type="checkbox"/> Load 200 µl of library into the inlet port using a P1000 pipette. <input type="checkbox"/> Close the valve to seal the inlet port. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output. 	
<p>If the light shield has been removed from the flow cell, install the light shield as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Align the inlet port cut out of the light shield with the inlet port cover on the flow cell. The leading edge of the light shield should sit above the flow cell ID. <input type="checkbox"/> Firmly press the light shield around the inlet port cover. The inlet port clip will click into place underneath the inlet port cover. 	

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Close the PromethION lid when ready to start a sequencing run on MinKNOW.	
Washing and reloading a PromethION Flow Cell	
<p>We recommend to wash the flow cell when ~25% starting pore count is remaining.</p> <p><input type="checkbox"/> Place the tube of Wash Mix (WMX) on ice. Do not vortex the tube.</p> <p><input type="checkbox"/> Thaw one tube of Wash Diluent (DIL) at RT.</p> <p><input type="checkbox"/> Mix the contents of Wash Diluent (DIL) thoroughly by vortexing, then spin down briefly and place on ice.</p> <p>In a clean 1.5 ml Eppendorf DNA LoBind tube, prepare the following Flow Cell Wash Mix:</p> <p><input type="checkbox"/> 2 µl Wash Mix (WMX)</p> <p><input type="checkbox"/> 398 µl Wash Diluent (DIL)</p> <p><input type="checkbox"/> Mix well by pipetting, and place on ice. Do not vortex the tube.</p> <p><input type="checkbox"/> Pause the sequencing experiment in MinKNOW, and leave the flow cell in the device.</p>	
IMPORTANT	
<p><input type="checkbox"/> It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.</p>	
<p>Remove waste buffer, as follows:</p> <p><input type="checkbox"/> Close the inlet port.</p> <p><input type="checkbox"/> Insert a P1000 pipette into a waste port and remove the waste buffer.</p> <p><input type="checkbox"/> Slide the inlet port cover clockwise to open the inlet port.</p>	
IMPORTANT	
<p><input type="checkbox"/> 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.</p>	
<p>After opening the inlet port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:</p> <p><input type="checkbox"/> Set a P1000 pipette to 200 µl</p> <p><input type="checkbox"/> Insert the tip into the inlet 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>Slowly load 200 µl of the prepared flow cell wash mix into the inlet port, as follows:</p> <p><input type="checkbox"/> Using a P1000 pipette, take 200 µl of the flow cell wash mix</p> <p><input type="checkbox"/> Insert the pipette tip into the inlet port, ensuring there are no bubbles in the tip</p> <p><input type="checkbox"/> Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip.</p> <p><input type="checkbox"/> Set a timer for a 5 minute incubation.</p>	

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<p>Once the 5 minute incubation time is complete, carefully load the remaining 200 µl of the prepared flow cell wash mix into the inlet port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Using a P1000 pipette, take 200 µl of the flow cell wash mix <input type="checkbox"/> Insert the pipette tip into the inlet port, ensuring there are no bubbles in the tip <input type="checkbox"/> Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip. <input type="checkbox"/> Close the inlet port and wait for 1 hour. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels. 	
<p>Remove the waste buffer, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Ensure the inlet port is closed. <input type="checkbox"/> Insert a P1000 pipette into a waste port and remove the waste buffer 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> The buffers used in this process are incompatible with conducting a Flow Cell Check step prior to loading the subsequent library. However, number of available pores will be reported after the next pore scan. 	
<ul style="list-style-type: none"> <input type="checkbox"/> Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT, before mixing by vortexing. Then spin down before storing on ice. <p>Prepare the flow cell priming mix in a suitable tube for the number of flow cells to flush. Once combined, mix well by briefly vortexing.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 1,170 µl Flow Cell Flush (FCF) <input type="checkbox"/> 30 µl Flow Cell Tether (FCT) <input type="checkbox"/> Slide the inlet port cover clockwise to open. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> 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. 	
<p>After opening the inlet port, draw back a small volume to remove any air bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette tip to 200 µl. <input type="checkbox"/> Insert the tip into the inlet port. <input type="checkbox"/> Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip. <p>Slowly load 500 µl of the priming mix into the inlet port, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Using a P1000 pipette, take 500 µl of the priming mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip. 	
<p>IMPORTANT</p> <ul style="list-style-type: none"> <input type="checkbox"/> It is vital to wait five minutes between the priming mix flushes to ensure effective removal of the nuclease. 	

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<input type="checkbox"/> Close the inlet port and wait five minutes. <input type="checkbox"/> Thoroughly mix the contents of the Library Beads (LIB) by pipetting.	
IMPORTANT <input type="checkbox"/> The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	
In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows: <input type="checkbox"/> 100 µl Sequencing Buffer (SB) <input type="checkbox"/> 68 µl Library Beads (LIB) thoroughly mixed before use, or Library Solution (LIS) <input type="checkbox"/> 32 µl DNA library	
IMPORTANT <input type="checkbox"/> It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
Remove the waste buffer, as follows: <input type="checkbox"/> Ensure the inlet port is closed. <input type="checkbox"/> Insert a P1000 pipette into a waste port and remove the waste buffer <input type="checkbox"/> Slide the inlet port cover clockwise to open.	
IMPORTANT <input type="checkbox"/> 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 inlet port, draw back a small volume to remove any air bubbles: <input type="checkbox"/> Set a P1000 pipette tip to 200 µl. <input type="checkbox"/> Insert the tip into the inlet port. <input type="checkbox"/> Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip. Slowly load 500 µl of the priming mix into the inlet port, as follows: <input type="checkbox"/> Using a P1000 pipette, take 500 µl of the priming mix <input type="checkbox"/> Insert the pipette tip into the priming port, ensuring there are no bubbles in the tip <input type="checkbox"/> Slowly twist the pipette wheel down to load the flow cell (if possible with your pipette) or push down the plunger very slowly, leaving a small volume of buffer in the pipette tip.	
IMPORTANT <input type="checkbox"/> It is vital that the inlet port is closed before removing waste to prevent air from being drawn across the sensor array area, which would lead to a significant loss of sequencing channels.	
Remove waste buffer, as follows: <input type="checkbox"/> Close the inlet port. <input type="checkbox"/> Insert a P1000 pipette into a waste port and remove the waste buffer. <input type="checkbox"/> Slide the inlet port cover clockwise to open.	

Ligation Sequencing gDNA V14 - human sample (N50 30 kb) on PromethION (SQK-LSK114)

Version: GDH_9174_v114_revN_10Nov2022
Last update: 29/02/2024



Flow Cell Number:

DNA Samples:

INSTRUCTIONS	NOTES/OBSERVATIONS
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p> <p>After opening the inlet port, draw back a small volume to remove any air bubbles:</p> <p><input type="checkbox"/> Set a P1000 pipette tip to 200 µl.</p> <p><input type="checkbox"/> Insert the tip into the inlet port.</p> <p><input type="checkbox"/> Turn the wheel until the dial shows 220-230 µl, or until you see a small volume of buffer entering the pipette tip.</p> <p><input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading.</p> <p><input type="checkbox"/> Load 200 µl of library into the inlet port using a P1000 pipette.</p> <p><input type="checkbox"/> Close the valve to seal the inlet port.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.</p> <p>If the light shield has been removed from the flow cell, install the light shield as follows:</p> <p><input type="checkbox"/> Align the inlet port cut out of the light shield with the inlet port cover on the flow cell. The leading edge of the light shield should sit above the flow cell ID.</p> <p><input type="checkbox"/> Firmly press the light shield around the inlet port cover. The inlet port clip will click into place underneath the inlet port cover.</p> <p><input type="checkbox"/> Close the PromethION lid when ready to start a sequencing run on MinKNOW.</p>	
<p>Repeat the "Washing and reloading a PromethION flow cell" step twice for a total of three library loads to maximise data acquisition.</p>	
<p>Flow cell reuse and returns</p>	
<p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	