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

Before start checklist

Materials

- [ ] 50 ng high molecular weight genomic DNA per sample
- [ ] For R10.3 flow cells, 50-200 ng high molecular weight genomic DNA per sample
- [ ] Rapid Barcoding Kit 96 (SQK-RBK110.96)

Consumables

- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 2 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Cat # 0030129504) with heat seals
- [ ] Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- [ ] Freshly-prepared 80% ethanol in nuclease-free water

Equipment

- [ ] Ice bucket with ice
- [ ] Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, #11766427)
- [ ] Timer
- [ ] Thermal cycler or heat block at 30°C and 80°C
- [ ] Magnetic rack
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Pipettes and pipette tips P2, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	

- [ ] Program the thermal cycler: 30°C for 2 minutes, then 80°C for 2 minutes.

Thaw kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:

- [ ] Rapid Barcode plate (RB96): not frozen, briefly spin down, mix well by pipetting
- [ ] Rapid Adapter F (RAP-F): not frozen, briefly spin down, mix well by pipetting
- [ ] SPRI beads (SPRI): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use
- [ ] Sequencing Buffer II (SBII): thaw at RT, briefly spin down, mix well by pipetting*
- [ ] Loading Beads II (LBII): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use
- [ ] Elution Buffer (EB): thaw at RT, briefly spin down, mix well by pipetting
- [ ] Flush Buffer (FB): thaw at RT, briefly spin down, mix by vortexing
- [ ] Flush Tether (FLT): thaw at RT, briefly spin down, mix well by pipetting

Prepare the DNA in Nuclease-free water.

- [ ] Transfer 50 ng genomic DNA per sample (if using R9.4.1 flow cells) or 50-200 ng (if using R10.3 flow cells) into a 1.5 ml Eppendorf DNA LoBind tube
- [ ] Adjust the volume to 9 µl with Nuclease-free water
- [ ] Mix by pipetting up and down
- [ ] Spin down briefly in a microfuge

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In 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind, mix the following. The Rapid Barcodes can be transferred using a multichannel pipette: ☐ 9 µl 50 ng template DNA ☐ 1 µl Rapid Barcodes (RB01-96, one for each sample) ☐ Mix well by pipetting. Seal the plate and spin down in a centrifuge. ☐ Incubate the tubes or plate at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tubes or plate on ice to cool. ☐ Pool all barcoded samples in your desired ratio, noting the total volume. ☐ Resuspend the SPRI beads by vortexing. ☐ To the entire pooled barcoded sample from Step 7, add an equal volume of resuspended SPRI beads and mix by flicking the tube. ☐ Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT. ☐ Prepare at least 3 ml of fresh 80% ethanol in Nuclease-free water. ☐ Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant. ☐ Keep the tube on the magnet and wash the beads with 1.5 ml of freshly-prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. ☐ Repeat the previous step. ☐ Briefly 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. ☐ Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB). Incubate for 10 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless. Remove and retain 15 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Remove and retain the eluate which contains the DNA library in a clean 1.5 ml Eppendorf DNA LoBind tube ☐ Dispose of the pelleted beads ☐ Quantify 1 µl of eluted sample using a Qubit fluorometer. ☐ Transfer 11 µl of the sample into a clean 1.5 ml Eppendorf DNA LoBind tube. ☐ Add 1 µl of Rapid Adapter F (RAP-F) to 11 µl of barcoded DNA. ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the reaction for 5 minutes at RT.	
The prepared library is used for loading into the MinION flow cell. Store the library on ice until ready to load.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Priming and loading the SpotON Flow Cell	
Using the Loading Solution ☐ Thaw the Sequencing Buffer II (SBII), Loading Beads II (LBII) or Loading Solution (LS, if using), Flush Tether (FLT) and Flush Buffer (FB) at RT. ☐ Mix the Sequencing Buffer II (SBII), Flush Buffer (FB) and Flush Tether (FLT) tubes by vortexing. Spin down the SBII and FLT at RT. ☐ Open the MinION Mk1B lid and slide the flow cell under the clip. ☐ Slide the priming port cover clockwise to open the priming port.	
IMPORTANT ☐ 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 priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles (a few µl): ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the priming port ☐ Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip ☐ To prepare the flow cell priming mix, add 30 µl of thawed and mixed Flush Tether (FLT) to 1.17 ml of thawed and mixed Flush Buffer (FB), and mix by vortexing at RT. ☐ 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. During this time, prepare the library for loading by following the steps below. ☐ Thoroughly mix the contents of the Loading Beads II (LBII) by pipetting.	
IMPORTANT ☐ The Loading Beads II (LBII) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.	
In a new tube, prepare the library for loading as follows: ☐ 37.5 µl Sequencing Buffer II (SBII) ☐ 25.5 µl Loading Beads II (LBII) mixed immediately before use, or Loading Solution (LS), if using ☐ 12 µl DNA library Complete the flow cell priming: ☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible. ☐ Load 200 µl of the priming mix into the flow cell via the priming port (not the SpotON sample port), avoiding the introduction of air bubbles. ☐ Mix the prepared library gently by pipetting up and down just prior to loading. ☐ Add 75 µl of sample to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION Mk1B lid.	
Ending the experiment	
☐ After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Wash Kit instructions and store the washed flow cell at 2-8°C, OR ☐ Follow the returns procedure by washing out the flow cell ready to send back to Oxford Nanopore.	
IMPORTANT ☐ 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.	