Rapid sequencing DNA V14 - barcoding (SQK-RBK114.24 or SQK-RBK114.96)

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

Before start checklist

Materials

- [ ] 200 ng gDNA per sample
- [ ] Rapid Barcoding Kit 24 V14 (SQK-RBK114.24) OR Rapid Barcoding Kit 96 V14 (SQK-RBK114.96)

Consumables

- [ ] MinION and GridION Flow Cell
- [ ] Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)
- [ ] Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)
- [ ] Freshly prepared 80% ethanol in nuclease-free water
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Eppendorf™, cat # 0030129504) with heat seals
- [ ] 2 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] 1.5 ml Eppendorf DNA LoBind tubes

Equipment

- [ ] MinION or GridION device
- [ ] MinION and GridION Flow Cell Light Shield
- [ ] Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific, 11766427)
- [ ] Timer
- [ ] Thermal cycler or heat blocks
- [ ] Magnetic rack
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Ice bucket with ice
- [ ] Qubit fluorometer (or equivalent for QC check)
- [ ] Pipettes and pipette tips Multichannel, P2, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	
Minimum Rapid Barcode use requirements	
Check your flow cell.	
- [ ] 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 Barcodes (RB01-24) or Rapid Barcode Plate (RB01-96): not frozen, briefly spin down, mix well by pipetting - [ ] Rapid Adapter (RA): not frozen, briefly spin down, mix well by pipetting - [ ] AMPure XP Beads (AXP): 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 - [ ] Adapter Buffer (ADB): thaw at RT, briefly spin down, mix by vortexing	

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INSTRUCTIONS	NOTES/OBSERVATIONS

Prepare the DNA in Nuclease-free water.

- ☐ Transfer 200 ng of genomic DNA per sample into 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind.
- ☐ Adjust the volume of each sample to 10 µl with Nuclease-free water.
- ☐ Pipette mix the tubes for 10-15 times to avoid unwanted shearing
- ☐ Spin down briefly in a microfuge

In the 0.2 ml thin-walled PCR tubes or an Eppendorf twin.tec® PCR plate 96 LoBind, mix the following:

- ☐ 10 µl Template DNA (200 ng from previous step)
- ☐ 1.5 µl Rapid Barcodes (RB01-24 or RB01-96, one for each sample)

☐ Ensure the components are thoroughly mixed by pipetting and spin down briefly.

☐ 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.

☐ Spin down the tubes or plate to collect the liquid at the bottom.

☐ Pool all barcoded samples in a clean 2 ml Eppendorf DNA LoBind tube, noting the total volume.

☐ Resuspend the AMPure XP Beads (AXP) by vortexing.

IMPORTANT

☐ Ensure you have sufficient capacity in your reaction tube for all the reagents.

Add an equal volume of resuspended AMPure XP Beads (AXP) to the entire pooled barcoded sample, and mix by flicking the tube.

- ☐ Volume of AMPure XP Beads (AXP) added
  - Volume per sample: 11.5 µl
  - For 4 samples: 46 µl
  - For 12 samples: 138 µl
  - For 24 samples: 276 µl
  - For 48 samples: 552 µl
  - For 96 samples: 1,000 µl

☐ Incubate on a Hula mixer (rotator mixer) for 10 minutes at RT.

☐ Prepare at least 2 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 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.

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Remove the tube from the magnetic rack and resuspend the pellet in 15 µl Elution Buffer (EB) per 24 barcodes used. ☐ Volume of Elution Buffer (EB) - For 24 barcodes: 15 µl - For 48 barcodes: 30 µl - For 72 barcodes: 45 µl - For 96 barcodes: 60 µl ☐ Incubate for 10 minutes at RT. ☐ Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute. Remove and retain the full volume 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. In a fresh 1.5 ml Eppendorf DNA LoBind tube, dilute the Rapid Adapter (RA) as follows and pipette mix: ☐ 1.5 µl Rapid Adapter (RA) ☐ 3.5 µl Adapter Buffer (ADB) ☐ Add 1 µl of the diluted Rapid Adapter (RA) to the 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 flow cell. Store the library on ice until ready to load.	
Priming and loading the MinION and GridION Flow Cell	
IMPORTANT ☐ Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).	
☐ 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.	
IMPORTANT ☐ For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.	
To prepare the flow cell priming mix with BSA, combine the following reagents in a fresh 1.5 ml Eppendorf DNA LoBind tube. Mix by inverting the tube and pipette mix at RT: ☐ 1,170 µl Flow Cell Flush (FCF) ☐ 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml ☐ 30 µl Flow Cell Tether (FCT) ☐ 1,205 µl Final total volume in tube	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact. ☐ Slide the flow cell 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: ☐ Set a P1000 pipette to 200 µl ☐ Insert the tip into the priming port ☐ Turn the wheel until the dial shows 220-230 µl, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip Note: Visually check that there is continuous buffer from the priming port across the sensor array. ☐ Load 800 µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles. Wait for five minutes. During this time, prepare the library for loading by following the steps below. ☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting.	
IMPORTANT ☐ 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: ☐ 37.5 µl Sequencing Buffer (SB) ☐ 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), 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 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 the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next. ☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.	
IMPORTANT ☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.	

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Place the light shield onto the flow cell, as follows: ☐ Carefully place the leading edge of the light shield against the clip. Note: Do not force the light shield underneath the clip. ☐ Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.	
Close the device lid and set up a sequencing run on MinKNOW.	
Flow cell reuse and returns	
☐ 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. ☐ Alternatively, follow the returns procedure to flush 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.	