Rapid sequencing gDNA - Field Sequencing Kit (SQK-LRK001)

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

Before start checklist

Materials

- [ ] ~400 ng high molecular weight genomic DNA per sample
- [ ] Field Sequencing Kit (SQK-LRK001)
- [ ] Flow Cell Priming Kit (EXP-FLP001)

Consumables

Equipment

- [ ] Timer
- [ ] Method of achieving 80° C
- [ ] Pipettes and pipette tips P10, P20, P100, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	
DNA tagmentation Prepare the DNA in Nuclease-free water. ☐ Transfer ~400 ng genomic DNA into a DNA LoBind tube ☐ Adjust the volume to 10 µl with Nuclease-free water ☐ Mix by flicking the tube to avoid unwanted shearing ☐ Spin down briefly in a microfuge ☐ Cut off Tube 1 (left hand tube, containing FRL) ☐ Using a clean, empty pipette tip, pierce the foil of Tube 1. Take care to not disturb the pellet. ☐ Add 10 µl of the input DNA to Tube 1. ☐ Mix gently by pipetting up and down. Make sure all liquid is collected at the bottom of the tube. ☐ Incubate the tube at RT for 1 minute and then at 80° C for 1 minute. Adapter attachment ☐ Using a clean empty pipette tip, pierce the foil of Tube 2. Take care to not disturb the pellet. ☐ Transfer 10 µl of the tagmented DNA from Tube 1 to Tube 2. ☐ Mix gently by pipetting up and down. Make sure all liquid is collected at the bottom of the tube. ☐ Incubate the reaction for 5 minutes at RT. ☐ Using a clean empty pipette tip, pierce the foil of Tube 3 (right-hand tube). Take care to not disturb the pellet. ☐ Add 65 µl Resuspension Buffer (RTB) into Tube 3. Mix by pipetting up and down, and make sure all liquid is collected at the bottom of the tube.	
The prepared DNA library is used for loading into the flow cell.	

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Priming and loading the SpotON flow cell	
☐ Mix the Flush Tether (FLT) and Flush Buffer (FLB) tubes by pipetting and spin down 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: ☐ 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. ☐ Prepare the priming mix: add 30 µl of Flush Tether to the tube of Flush Buffer, and mix well by pipetting up and down. ☐ 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. ☐ Prepare the library for loading: add 65 µl of the resuspended Tube 3 material into Tube 2. Mix by pipetting up and down. 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. ☐ Add 75 µl of the sample (mix from Tube 2 and Tube 3) 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, close the priming port and replace the MinION device lid.	
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.	

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☐ 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.	