Rapid Lambda Control Experiment (SQK-RAD004)

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

Before start checklist

- [ ] Rapid Sequencing Kit (SQK-RAD004)
- [ ] Flow Cell Priming Kit (EXP-FLP001)
- [ ] Microfuge
- [ ] Timer
- [ ] Method of heating to 80° C for 1 minute
- [ ] Pipettes and pipette tips P2, P10, P20, P100, P1000
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] Nuclease-free water

INSTRUCTIONS	NOTES/OBSERVATIONS
Check your flow cell	
- [ ] Set up the MinION, Flow Cell and host computer Once successfully plugged in, you will see a light and hear the fan. Open the MinKNOW GUI from the desktop icon and establish a local or remote connection. - [ ] If running a MinION on the same host computer, plug the MinION into the computer. When the connection name appears under the Local tab, click Connect. - [ ] If running a MinION on a remote computer, first enter the name or IP address of the remote host under Remote and click Connect. - [ ] Plug a MinION and Flow Cell into the remote computer; the connection IDs will be displayed under MinION Connection and Flowcell Connection. Enter the SampleID and FlowcellID being used, and click Submit. - [ ] Once a MinION and Flow Cell are connected, a Label Experiment dialogue box appears. - [ ] Click into the Sample ID box and name your sample using free text in alphanumeric format only, deleting any default Sample_ID that is present. Warning: SampleID should not contain any personally identifiable information. - [ ] Click into the FlowcellID box and enter the Flow Cell ID, which is the code found on a sticker on the top side of a Flow Cell. - [ ] Select the Platform QC script under Choose Operation, and start the script using the Execute button. - [ ] Check the number of active pores available for the experiment, reported in the message panel or in notifications when the check is complete.	
Flow cell check complete.	
Library preparation	
DNA tagmentation	LMD

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Thaw kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below: ☐ Lambda DNA (LMD): thaw at RT, briefly spin down, mix well by pipetting ☐ Fragmentation Mix (FRA): not frozen, briefly spin down, mix well by pipetting ☐ Rapid Adapter (RAP): not frozen, briefly spin down, mix well by pipetting ☐ Sequencing Buffer (SQB): thaw at RT, briefly spin down, mix well by pipetting* ☐ Loading Beads (LB): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use ☐ Flush Buffer (FLB): thaw at RT, briefly spin down, mix well by pipetting* ☐ Flush Tether (FLT): thaw at RT, briefly spin down, mix well by pipetting ☐ Once thawed, keep all the kit components on ice. In a 0.2 ml thin-walled PCR tube, mix the following: ☐ 7.5 µl Lambda DNA ☐ 2.5 µl FRA ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the tube at 30° C for 1 minute and then at 80° C for 1 minute.	
375 ng tagmented Lambda DNA in 10 µl is taken into the next step.	
Adapter attachment ☐ Add 1 µl of RAP to the tube. ☐ Mix gently by flicking the tube, and spin down. ☐ Incubate the reaction for 5 minutes at RT.	
The prepared DNA library is used for loading into the flow cell. Store the library on ice until ready to load.	
Priming and loading the SpotON Flow Cell	
☐ 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.	
IMPORTANT ☐ 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.	
After opening the priming port, check for small bubble under the cover. Draw back a small volume to remove any bubble (a few µls): ☐ 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 ☐ 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.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
☐ 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. ☐ Thoroughly mix the contents of the SQB and LB tubes by pipetting. In a new tube, prepare the library for loading as follows: ☐ 34 µl Sequencing Buffer (SQB) ☐ 25.5 µl Loading Beads (LB) ☐ 4.5 µl Nuclease-free water ☐ 11 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. ☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the priming port and replace the MinION lid.	
Onward analysis of MinKNOW basecalled data	
☐ Open the Desktop Agent using the desktop shortcut. ☐ Click on the New Workflow tab in the Desktop Agent and select the Control Experiment workflow. ☐ Check the correct settings are selected in the Desktop Agent. ☐ Click "Start Run" to start data analysis. ☐ Follow the progression of upload and download of read files in the Desktop Agent, along with network speed. Click on VIEW REPORT. ☐ Click on VIEW REPORT to navigate to the Metrichor website, this can be done at any point during data exchange ☐ Return to the Desktop Agent to see progression of the exchange	
Close down MinKNOW and the Desktop Agent	
☐ Quit Desktop Agent using the close x. ☐ Quit MinKNOW by closing down the web GUI. ☐ Disconnect the MinION.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
Prepare the flow cell for re-use or return to Oxford Nanopore.	
☐ 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 ☐ Follow the returns procedure by washing out the MinION Flow Cell ready to send back to Oxford Nanopore.	