Rapid sequencing gDNA - whole genome amplification (SQK-RAD004)

Version: RWGA_9041_v1_revS_14Aug2019
Last update: 07/09/2023

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

Before start checklist

Materials

- ☐ >300 cells in 0.5 µl nuclease-free water, or 1-10 ng DNA in 1 µl nuclease-free water
- ☐ Qiagen REPLI-g UltraFast Mini Kit
- ☐ Rapid Sequencing Kit (SQK-RAD004)
- ☐ Flow Cell Priming Kit (EXP-FLP002)

Consumables

- ☐ 1.5 ml Eppendorf DNA LoBind tubes
- ☐ 0.2 ml thin-walled PCR tubes
- ☐ Nuclease-free water (e.g. ThermoFisher, AM9937)
- ☐ Phosphate buffered saline (PBS)
- ☐ Pipette tips P2, P10, P20, P100, P200, P1000

Equipment

- ☐ Microfuge
- ☐ Vortex mixer
- ☐ Method of heating to 80° C for 1 minute
- ☐ Ice bucket with ice
- ☐ Timer
- ☐ Pipettes P2, P10, P20, P100, P200, P1000

INSTRUCTIONS	NOTES/OBSERVATIONS
Library preparation	
Input recommendation If starting with whole cells, follow the instructions below. In a 0.2 ml thin-walled PCR tube, mix together in the following order: ☐ 0.5 µl Cell sample ☐ 1 µl Phosphate buffered saline (PBS) ☐ 1.5 µl Reagent D2 from the REPLI-g UltraFast Mini Kit ☐ Incubate the reaction for 5 minutes at RT. ☐ Add 1.5 µl Stop Reagent. In a 0.2 ml thin-walled PCR tube, mix together in the following order: ☐ 15 µl REPLI-g UltraFast Reaction Buffer ☐ 1 µl REPLI-g UltraFast Reaction Polymerase ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Add the lysed cells from the previous step to the tube. ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the reaction at 30° C until the DNA concentration is > 80 ng/µl. Quantification should be performed using the Qubit dsDNA BR Assay Kit. If starting with purified DNA, follow the instructions below.	FRA

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☐ Put the DNA in 1 µl into a clean tube. ☐ Add 1 µl Buffer D1 to the tube. Mix by vortexing and spin down. ☐ Incubate for 3 minutes at RT. ☐ Add 2 µl Buffer N1 to the tube. Mix by vortexing and spin down. In a 0.2 ml thin-walled PCR tube, mix together in the following order: ☐ 15 µl REPLI-g UltraFast Reaction Buffer ☐ 1 µl REPLI-g UltraFast Reaction Polymerase ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Add the DNA from the previous step to the tube. ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the reaction at 30° C until the DNA concentration is > 80 ng/µl. Quantification should be performed using the Qubit dsDNA BR Assay Kit. Regardless of input material, continue with the protocol as follows: In a 0.2 ml thin-walled PCR tube, mix the following: ☐ 2.5 µl Amplified DNA ☐ 5 µl Nuclease-free water ☐ 2.5 µl Fragmentation Mix (FRA) ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the tube at 30°C for 1 minute and then at 80°C for 1 minute. Briefly put the tube on ice to cool it down. ☐ Add 1 µl RAP to the 10 µl amplified DNA library. ☐ Ensure the components are thoroughly mixed by pipetting, and spin down. ☐ Incubate the reaction for 5 minutes at RT.	
The prepared library is used for loading onto the flow cell. Store the library on ice until ready to load.	
Priming and loading the SpotON flow cell	
☐ Thaw the Sequencing Buffer (SQB), Loading Beads (LB), Flush Tether (FLT) and one tube of Flush Buffer (FB) at RT before mixing the reagents by vortexing, and spin down at RT. ☐ To 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 (FB), and mix by vortexing at RT. ☐ Open the MinION device lid and slide the flow cell under the clip.	

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☐ 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. ☐ 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 Loading Beads (LB) tubes by vortexing.	
IMPORTANT ☐ The Loading Beads (LB) 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: ☐ 34 µl Sequencing Buffer (SQB) ☐ 25.5 µl Loading Beads (LB), mixed immediately before use ☐ 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 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.	
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.	

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INSTRUCTIONS	NOTES/OBSERVATIONS
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.	