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

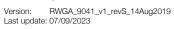




Before start checklist		
Materials	Consumables	Equipment
>300 cells in 0.5 µl nuclease-free water, or 1- 10 ng DNA in 1 µl nuclease-free water	1.5 ml Eppendorf DNA LoBind tubes	Microfuge
Qiagen REPLI-g UltraFast Mini Kit	0.2 ml thin-walled PCR tubes	☐ Vortex mixer
Rapid Sequencing Kit (SQK-RAD004)	Nuclease-free water (e.g. ThermoFisher, AM9937)	Method of heating to 80° C for 1 minute
Flow Cell Priming Kit (EXP-FLP002)	Phosphate buffered saline (PBS)	lce bucket with ice
	Pipette tips P2, P10, P20, P100, P200, P1000	Timer
		Pipettes P2, P10, P20, P100, P200, P1000
INSTRUCTIONS		NOTES/OBSERVATIONS
Library preparation		
Input recommendation		FRA
If starting with whole cells, follow the instructions be	elow.	
In a 0.2 ml thin-walled PCR tube, mix together in the 0.5 µl Cell sample 1 µl Phosphate buffered saline (PBS) 1.5 µl Reagent D2 from the REPLI-g UltraFast		
☐ 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 15 µl REPLI-g UltraFast Reaction Buffer 1 µl REPLI-g UltraFast Reaction Polymerase	e following order:	
☐ Ensure the components are thoroughly mixed by	y pipetting, and spin down.	
Add the lysed cells from the previous step to the	tube.	
☐ Ensure the components are thoroughly mixed b	y pipetting, and spin down.	
☐ Incubate the reaction at 30° C until the DNA cor performed using the Qubit dsDNA BR Assay Kit	ncentration is $>$ 80 ng/ μ l. Quantification should be .	
If starting with purified DNA, follow the instructions by	pelow.	

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low Cell Number:	DNA Samples:

INSTRUCTIONS	NOTES/OBSERVATIONS
Put the DNA in 1 µl into a clean tube.	
$\hfill \square$ Add 1 μI Buffer D1 to the tube. Mix by vortexing and spin down.	
☐ Incubate for 3 minutes at RT.	
$\hfill \square$ Add 2 μI 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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INSTRUCTIONS	NOTES/OBSERVATIONS
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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Flow Cell Number:	DNA Samples:

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.	

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