

Rapid sequencing gDNA - barcoding (SQK-RBK004)

Version: RBK_9054_v2_revAF_14Aug2019
 Last update: 07/09/2023



Flow Cell Number:

DNA Samples:

Before start checklist		
Materials	Consumables	Equipment
<input type="checkbox"/> ~400 ng high molecular weight genomic DNA per sample	<input type="checkbox"/> 1.5 ml Eppendorf DNA LoBind tubes	<input type="checkbox"/> Ice bucket with ice
<input type="checkbox"/> Rapid Barcoding Sequencing Kit (SQK-RBK004)	<input type="checkbox"/> 0.2 ml thin-walled PCR tubes	<input type="checkbox"/> Microfuge
<input type="checkbox"/> Flow Cell Priming Kit (EXP-FLP002)	<input type="checkbox"/> Nuclease-free water (e.g. ThermoFisher, AM9937)	<input type="checkbox"/> Timer
	<input type="checkbox"/> Agencourt AMPure XP beads (optional) (Beckman Coulter™ cat # A63881)	<input type="checkbox"/> Thermal cycler or heat blocks
	<input type="checkbox"/> Freshly prepared 70% ethanol in nuclease-free water (optional)	<input type="checkbox"/> Pipettes and pipette tips P2, P20, P100, P200, P1000
	<input type="checkbox"/> 10 mM Tris-HCl pH 8.0 with 50 mM NaCl (optional)	

INSTRUCTIONS	NOTES/OBSERVATIONS
<p>Library preparation</p> <p>Thaw kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Fragmentation Mix RB01-12: not frozen, briefly spin down, mix well by pipetting <input type="checkbox"/> Rapid Adapter (RAP): not frozen, briefly spin down, mix well by pipetting <input type="checkbox"/> Sequencing Buffer (SQB): thaw at RT, briefly spin down, mix well by pipetting* <input type="checkbox"/> Loading Beads (LB): thaw at RT, briefly spin down, mix by pipetting or vortexing immediately before use <input type="checkbox"/> Flush Buffer (FLB) - 1 tube: thaw at RT, briefly spin down, mix well by pipetting* <input type="checkbox"/> Flush Tether (FLT): thaw at RT, briefly spin down, mix well by pipetting <p>Prepare the DNA in Nuclease-free water.</p> <ul style="list-style-type: none"> <input type="checkbox"/> Transfer ~400 ng genomic DNA into a DNA LoBind tube <input type="checkbox"/> Adjust the volume to 7.5 µl with Nuclease-free water <input type="checkbox"/> Mix by flicking the tube to avoid unwanted shearing <input type="checkbox"/> Spin down briefly in a microfuge <p>In a 0.2 ml thin-walled PCR tube, mix the following:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 7.5 µl 400 ng template DNA <input type="checkbox"/> 2.5 µl Fragmentation Mix RB01-12 (one for each sample) <p><input type="checkbox"/> Mix gently by flicking the tube, and spin down.</p> <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Pool the barcoded samples in a 1.5 ml Eppendorf DNA LoBind tube.</p>	

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<p>IMPORTANT</p> <p><input type="checkbox"/> If barcoding four or more samples, increased throughput can be achieved through cleaning up and concentrating the pooled material using AMPure XP beads as outlined in Steps 6-17. Otherwise, for a more rapid sample preparation, transfer 10 µl of pooled sample from Step 6 into a clean 1.5 ml Eppendorf DNA LoBind tube, and proceed directly to Step 18.</p> <p><input type="checkbox"/> Resuspend the AMPure XP beads by vortexing.</p> <p><input type="checkbox"/> To the entire pooled barcoded sample from Step 6, add an equal volume of resuspended AMPure XP beads, and mix by flicking the tube.</p> <p><input type="checkbox"/> Incubate on a Hula mixer (rotator mixer) for 5 minutes at RT.</p> <p><input type="checkbox"/> Prepare 500 µl of fresh 70% ethanol in Nuclease-free water.</p> <p><input type="checkbox"/> Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.</p> <p><input type="checkbox"/> Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 70% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.</p> <p><input type="checkbox"/> Repeat the previous step.</p> <p><input type="checkbox"/> Spin down and place the tube back on the magnet. Pipette off any residual 70% ethanol. Briefly allow to dry.</p> <p><input type="checkbox"/> Remove the tube from the magnetic rack and resuspend pellet in 10 µl of 10 mM Tris-HCl pH 8.0 with 50 mM NaCl. Incubate for 2 minutes at RT.</p> <p><input type="checkbox"/> Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.</p> <p>Remove and retain 10 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.</p> <p><input type="checkbox"/> Dispose of the pelleted beads</p> <p>End of optional steps.</p> <p><input type="checkbox"/> Add 1 µl of RAP to 10 µl of barcoded DNA.</p> <p><input type="checkbox"/> Mix gently by flicking the tube, and spin down.</p> <p><input type="checkbox"/> Incubate the reaction for 5 minutes at RT.</p>	
<p>The prepared library is used for loading into the flow cell. Store the library on ice until ready to load.</p>	
<p>Priming and loading the SpotON Flow Cell</p>	
<p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Open the MinION device lid and slide the flow cell under the clip.</p>	

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<p><input type="checkbox"/> Slide the priming port cover clockwise to open the priming port.</p>	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	
<p>After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Set a P1000 pipette to 200 µl <input type="checkbox"/> Insert the tip into the priming port <input type="checkbox"/> 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 <p>Note: Visually check that there is continuous buffer from the priming port across the sensor array.</p> <p><input type="checkbox"/> 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.</p> <p><input type="checkbox"/> Thoroughly mix the contents of the Loading Beads (LB) tubes by vortexing.</p> <p>In a new tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> 34 µl Sequencing Buffer (SQB) <input type="checkbox"/> 25.5 µl Loading Beads (LB), mixed immediately before use <input type="checkbox"/> 4.5 µl Nuclease-free water <input type="checkbox"/> 11 µl DNA library 	
<p>IMPORTANT</p> <p><input type="checkbox"/> 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.</p>	
<p>Complete the flow cell priming:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Gently lift the SpotON sample port cover to make the SpotON sample port accessible. <input type="checkbox"/> Load 200 µl of the priming mix into the flow cell priming port (not the SpotON sample port), avoiding the introduction of air bubbles. <input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading. <input type="checkbox"/> 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. <input type="checkbox"/> Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port. 	
<p>IMPORTANT</p> <p><input type="checkbox"/> Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.</p>	
<p>Place the light shield onto the flow cell, as follows:</p> <ul style="list-style-type: none"> <input type="checkbox"/> Carefully place the leading edge of the light shield against the clip. Note: Do not force the light shield underneath the clip. <input type="checkbox"/> 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. 	

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Close the device lid and set up a sequencing run on MinKNOW.	
Flow cell reuse and returns	
<input type="checkbox"/> 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. <input type="checkbox"/> Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore.	
IMPORTANT <input type="checkbox"/> 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.	