

# Rapid sequencing amplicons - 16S barcoding (SQK-16S024)

Version: 16S\_9086\_v1\_revZ\_14Aug2019  
 Last update: 06/12/2023



Flow Cell Number: .....

DNA Samples: .....

Before start checklist		
Materials	Consumables	Equipment
<input type="checkbox"/> 10 ng high molecular weight genomic DNA	<input type="checkbox"/> 1.5 ml Eppendorf DNA LoBind tubes	<input type="checkbox"/> Microfuge
<input type="checkbox"/> 16S Barcoding Kit 1-24 (SQK-16S024)	<input type="checkbox"/> 0.2 ml thin-walled PCR tubes	<input type="checkbox"/> Timer
<input type="checkbox"/> Flow Cell Priming Kit (EXP-FLP002)	<input type="checkbox"/> Nuclease-free water (e.g. ThermoFisher, AM9937)	<input type="checkbox"/> Thermal cycler
	<input type="checkbox"/> Agencourt AMPure XP beads (Beckman Coulter, A63881)	<input type="checkbox"/> Pipettes and pipette tips Multichannel, P2, P10, P20, P100, P200, P1000
	<input type="checkbox"/> LongAmp Hot Start Taq 2X Master Mix (NEB, M0533S)	
	<input type="checkbox"/> Freshly prepared 70% ethanol in nuclease-free water	
	<input type="checkbox"/> 10 mM Tris-HCl pH 8.0 with 50 mM NaCl	

INSTRUCTIONS	NOTES/OBSERVATIONS
<p><b>Library preparation</b></p> <p><input type="checkbox"/> Take one 96-well plate containing 16S barcodes. Break one set of barcodes (1-24, or as desired) away from the plate and return the rest to storage.</p> <p><b>IMPORTANT</b></p> <p><input type="checkbox"/> The 96-well plates are designed to break in one direction only. Strips, or multiple strips, of eight wells/barcodes can be removed from the plate at any one time.</p> <p><input type="checkbox"/> Thaw the desired barcodes, make sure the liquid is at the bottom of the tubes, and place on ice.</p> <p><input type="checkbox"/> Thaw the LongAmp Hot Start Taq 2X Master Mix, spin down briefly, mix well by pipetting and place on ice.</p> <p>Prepare the DNA in Nuclease-free water.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Transfer 10 ng genomic DNA into a DNA LoBind tube</li> <li><input type="checkbox"/> Adjust the volume to 10 µl with Nuclease-free water</li> <li><input type="checkbox"/> Mix thoroughly by flicking the tube, to avoid unwanted shearing</li> <li><input type="checkbox"/> Spin down briefly in a microfuge</li> </ul> <p>For each sample to be tested, prepare the following mixture in separate 0.2 ml thin-walled PCR tubes.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> 5 µl Nuclease-free water</li> <li><input type="checkbox"/> 10 µl Input DNA (10 ng)</li> <li><input type="checkbox"/> 25 µl LongAmp Hot Start Taq 2X Master Mix</li> </ul> <p><input type="checkbox"/> Ensure the components are thoroughly mixed by pipetting, and spin down.</p> <p><input type="checkbox"/> Using clean pipette tips, carefully pierce the foil surface of the required barcodes. Use a new tip for each barcode to avoid cross-contamination. Make a note of which barcode numbers will be run for each sample.</p>	

# Rapid sequencing amplicons - 16S barcoding (SQK-16S024)

Version: 16S\_9086\_v1\_revZ\_14Aug2019  
 Last update: 06/12/2023



Flow Cell Number: .....

DNA Samples: .....

INSTRUCTIONS	NOTES/OBSERVATIONS
<p><input type="checkbox"/> Using a multichannel pipette, mix the 16S barcodes by pipetting up and down 10 times. Transfer 10 µl of each 16S Barcode into respective sample-containing tubes.</p> <p><input type="checkbox"/> Mix thoroughly by pipetting up and down ten times.</p> <p>Amplify using the following cycling conditions:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Initial denaturation 1 min @ 95 °C (1 cycle)</li> <li><input type="checkbox"/> Denaturation 20 secs @ 95 °C (25 cycles)</li> <li><input type="checkbox"/> Annealing 30 secs @ 55 °C (25 cycles)</li> <li><input type="checkbox"/> Extension 2 mins @ 65 °C (25 cycles)</li> <li><input type="checkbox"/> Final extension 5 mins @ 65 °C (1 cycle)</li> <li><input type="checkbox"/> Hold @ 4 °C</li> </ul> <p><input type="checkbox"/> Transfer each sample to a separate 1.5 ml DNA LoBind Eppendorf tube. Carry out steps 11-21 for each sample, before pooling the samples at step 22.</p> <p><input type="checkbox"/> Resuspend the AMPure XP beads by vortexing.</p> <p><input type="checkbox"/> Add 30 µl of resuspended AMPure XP beads to the reaction and mix by pipetting.</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 when clear and colourless.</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 ethanol. Allow to dry for ~30 seconds, but do not dry the pellet to the point of cracking.</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.</p> <p>Remove and retain 10 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Dispose of the pelleted beads</li> </ul>	
<p>Quantify 1 µl of eluted sample using a Qubit fluorometer.</p>	
<ul style="list-style-type: none"> <li><input type="checkbox"/> Pool all barcoded libraries in the desired ratios to a total of 50-100 fmoles in 10 µl of 10 mM Tris-HCl pH 8.0 with 50 mM NaCl. For 16S amplicons of ~1500 bp, 50-100 fmoles equates to ~50-100 ng.</li> <li><input type="checkbox"/> Add 1 µl of RAP to the barcoded DNA.</li> <li><input type="checkbox"/> Mix gently by flicking the tube, and spin down.</li> <li><input type="checkbox"/> Incubate the reaction for 5 minutes at RT.</li> </ul>	

Rapid sequencing amplicons - 16S barcoding (SQK-16S024)

Version: 16S\_9086\_v1\_revZ\_14Aug2019  
 Last update: 06/12/2023



Flow Cell Number: .....

DNA Samples: .....

INSTRUCTIONS	NOTES/OBSERVATIONS
<p>The prepared library is used for loading into the flow cell. Store the library on ice until ready to load.</p>	
<p><b>Priming and loading the SpotON flow cell</b></p>	
<ul style="list-style-type: none"> <li><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.</li> <li><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.</li> <li><input type="checkbox"/> Open the MinION device lid and slide the flow cell under the clip.</li> <li><input type="checkbox"/> Slide the priming port cover clockwise to open the priming port.</li> </ul>	
<p><b>IMPORTANT</b></p> <ul style="list-style-type: none"> <li><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.</li> </ul>	
<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"> <li><input type="checkbox"/> Set a P1000 pipette to 200 µl</li> <li><input type="checkbox"/> Insert the tip into the priming port</li> <li><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</li> </ul> <p>Note: Visually check that there is continuous buffer from the priming port across the sensor array.</p> <ul style="list-style-type: none"> <li><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.</li> <li><input type="checkbox"/> Thoroughly mix the contents of the Loading Beads (LB) tubes by vortexing.</li> </ul>	
<p><b>IMPORTANT</b></p> <ul style="list-style-type: none"> <li><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.</li> </ul>	
<p>In a new tube, prepare the library for loading as follows:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> 34 µl Sequencing Buffer (SQB)</li> <li><input type="checkbox"/> 25.5 µl Loading Beads (LB), mixed immediately before use</li> <li><input type="checkbox"/> 4.5 µl Nuclease-free water</li> <li><input type="checkbox"/> 11 µl DNA library</li> </ul> <p>Complete the flow cell priming:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Gently lift the SpotON sample port cover to make the SpotON sample port accessible.</li> <li><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.</li> <li><input type="checkbox"/> Mix the prepared library gently by pipetting up and down just prior to loading.</li> </ul>	

# Rapid sequencing amplicons - 16S barcoding (SQK-16S024)

Version: 16S\_9086\_v1\_revZ\_14Aug2019  
Last update: 06/12/2023



Flow Cell Number: .....

DNA Samples: .....

INSTRUCTIONS	NOTES/OBSERVATIONS
<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.	
<b>Flow cell reuse and returns</b>	
<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.	
<b>IMPORTANT</b> <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.	