# Rapid Sequencing Kit V14 - Lambda control (SQK-RAD114)

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

## Before start checklist

### Materials

- [ ] Rapid Sequencing Kit V14 (SQK-RAD114)
- [ ] Control Expansion (EXP-CTL001)

### Consumables

- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] 0.2 ml thin-walled PCR tubes
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)

### Equipment

- [ ] Microfuge
- [ ] Timer
- [ ] Thermal cycler or heat blocks
- [ ] Pipettes and pipette tips P2, P10, P20, P100, P1000

| INSTRUCTIONS | NOTES/OBSERVATIONS |
|---|---|
| **Library preparation** | |
| DNA tagmentation<br><br>Thaw the kit components at RT, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:<br><br>☐ Once thawed, keep all the kit components on ice.<br><br>Prepare the Lambda DNA in Nuclease-free water.<br>☐ Transfer 2 µl Lambda DNA into a 1.5 ml Eppendorf DNA LoBind tube<br>☐ Adjust the volume to 10 µl with Nuclease-free water<br>☐ Mix by flicking the tube to avoid unwanted shearing<br>☐ Spin down briefly in a microfuge<br><br>In a 0.2 ml thin-walled PCR tube, mix the following:<br>☐ 10 Lambda DNA<br>☐ 1 Fragmentation Mix (FRA)<br><br>☐ Mix gently by flicking the tube, and spin down.<br><br>☐ Incubate the tube at 30°C for 2 minutes and then at 80°C for 2 minutes. Briefly put the tube on ice to cool it down. |  |
| The tagmented Lambda DNA in 11 µl is taken into the next step. | |
| Adapter attachment<br><br>In a fresh 1.5 ml Eppendorf DNA LoBind tube, dilute the Rapid Adapter (RA) as follows and pipette mix:<br>☐ 1.5 µl Rapid Adapter (RA)<br>☐ 3.5 µl Adapter Buffer (ADB)<br><br>☐ Add 1 µl of diluted Rapid Adapter (RA) to the tagmented DNA. | |

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| ☐ Mix gently by flicking the tube, and spin down.<br>☐ 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 MinION and GridION Flow Cell** | |
| IMPORTANT<br>☐ Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114). | |
| ☐ Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at RT before mixing by vortexing. Then spin down and store on ice. | |
| IMPORTANT<br>☐ For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml. | |
| To prepare the flow cell priming mix with BSA, combine Flow Cell Flush (FCF) and Flow Cell Tether (FCT), as directed below. Mix by pipetting at RT.<br>☐ 1,170 µl Flow Cell Flush (FCF)<br>☐ 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml<br>☐ 30 µl Flow Cell Tether (FCT) | |
| ☐ Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact. | |
| ☐ Slide the flow cell priming port cover clockwise to open the priming port. | |
| IMPORTANT<br>☐ 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:<br>☐ Set a P1000 pipette to 200 µl<br>☐ Insert the tip into the priming port<br>☐ 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<br>Note: Visually check that there is continuous buffer from the priming port across the sensor array.<br>☐ 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. | |
| IMPORTANT<br>☐ The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use. | |

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| ☐ Thoroughly mix the contents of the Library Beads (LIB) by pipetting.<br><br>In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:<br>☐ 37.5 µl Sequencing Buffer (SB)<br>☐ 25.5 µl Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using<br>☐ 12 µl DNA library<br><br>Complete the flow cell priming:<br>☐ Gently lift the SpotON sample port cover to make the SpotON sample port accessible.<br>☐ Load 200 µl of the priming mix into the flow cell priming port (not the SpotON sample port), avoiding the introduction of air bubbles.<br><br>☐ Mix the prepared library gently by pipetting up and down just prior to loading.<br><br>☐ 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.<br><br>☐ Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port. | |
| IMPORTANT<br>☐ Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output. | |
| Place the light shield onto the flow cell, as follows:<br>☐ Carefully place the leading edge of the light shield against the clip.<br>Note: Do not force the light shield underneath the clip.<br>☐ 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. | |
| Close the device lid and set up a sequencing run on MinKNOW. | |
| **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.<br><br>☐ Alternatively, follow the returns procedure to flush out the flow cell ready to send back to Oxford Nanopore. | |
| IMPORTANT<br>☐ 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. | |