Oxford Nanopore Technologies

MINION | GRIDION | FLONGLE | PROMETHION

Rapid Sequencing Kit V14 - gDNA (SQK-RAD114)

Overview

Before beginning, we strongly recommend that first-time users read and review the complete online protocol on the Nanopore Community which can be accessed via the QR code. The online protocol provides the full instructions, additional commentary, and considerations for various steps, as well as guidance on input amounts, DNA handling, and sequencing. **This reference card is designed for experienced users.**

1. Library preparation (~10 minutes)

1 **Check your flow cell.**

We recommend performing a flow cell check before starting your library prep to ensure you have a flow cell with enough pores for a good sequencing run.

See the flow cell check instructions in the MinKNOW protocol for more information.

2 **Thaw the kit components at room temperature, spin down briefly using a microfuge and mix by pipetting as indicated by the table below:**

Reagent	1. Thaw at room temperature	2. Briefly spin down	3. Mix well by pipetting
Fragmentation Mix (FRA)	Not frozen	✓	✓
Rapid Adapter (RA)	Not frozen	✓	✓
Adapter Buffer (ADB)	Not frozen	✓	✓

3 **Once thawed, keep all the kit components on ice.**

4 **Prepare the DNA in nuclease-free water.**

- Transfer 100-150 ng genomic DNA into a 1.5 ml Eppendorf DNA LoBind tube
- Adjust the volume to 10 µl with nuclease-free water
- Mix by flicking the tube to avoid unwanted shearing
- Spin down briefly in a microfuge

5 **In a 0.2 ml thin-walled PCR tube, mix the following:**

Reagent	Volume
100-150 ng template DNA	10 µl
Fragmentation Mix (FRA)	1 µl
Total	**11 µl**

6 **Mix gently by flicking the tube, and spin down.**

7 **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.**

8 **The tagmented DNA in 11 µl is taken into the adapter attachment step.**

9 **In a fresh 1.5 ml Eppendorf DNA LoBind tube, dilute the Rapid Adapter (RA) as follows and pipette mix:**

Reagents	Volume
Rapid Adapter (RA)	1.5 µl
Adapter Buffer (ADB)	3.5 µl
Total	**5 µl**

10 Add 1 µl of diluted Rapid Adapter (RA) to the tagmented DNA.

11 Mix gently by flicking the tube, and spin down.

12 Incubate the reaction for 5 minutes at room temperature.

13 The prepared DNA library is used for loading into the flow cell. Store the library on ice until ready to load.

2. Priming and loading the MinION and GridION Flow Cell (~5 minutes)

1 Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).

2 Priming and loading a flow cell

We recommend all new users watch the 'Priming and loading your flow cell' video before your first run.

3 Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at room temperature before mixing by vortexing. Then spin down and store on ice.

4 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.

Note: We do not recommend using any other albumin type (e.g. recombinant human serum albumin).

5 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 room temperature.

Note: We are in the process of reformatting our kits with single-use tubes into a bottle format. Please follow the instructions for your kit format.

Single-use tubes format: Add 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml and 30 µl Flow Cell Tether (FCT) directly to a tube of Flow Cell Flush (FCF).

Bottle format: In a suitable tube for the number of flow cells, combine the following reagents:

Reagent	Volume per flow cell
Flow Cell Flush (FCF)	1,170 µl
Bovine Serum Albumin (BSA) at 50 mg/ml	5 µl
Flow Cell Tether (FCT)	30 µl
Total volume	**1,205 µl**

6 Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the priming port cover to ensure correct thermal and electrical contact.

7 Complete a flow cell check to assess the number of pores available before loading the library.

This step can be omitted if the flow cell has been checked previously.

See the flow cell check instructions in the MinKNOW protocol for more information.

8 Slide the flow cell priming port cover clockwise to open the priming port.

9 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.

10 After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

1. Set a P1000 pipette to 200 µl
2. Insert the tip into the priming port
3. 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.

11 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.

12 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.

We recommend using the Library Beads (LIB) for most sequencing experiments. However, the Library Solution (LIS) is available for more viscous libraries.

13 Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

14 In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

Reagent	Volume per flow cell
Sequencing Buffer (SB)	37.5 µl
Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using	25.5 µl
DNA library	12 µl
Total	**75 µl**

15 Complete the flow cell priming:

1. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
2. Load **200 µl** of the priming mix into the flow cell priming port (**not** the SpotON sample port), avoiding the introduction of air bubbles.

16 Mix the prepared library gently by pipetting up and down just prior to loading.

17 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.

18 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port and close the priming port.

19 Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.

We recommend leaving the light shield on the flow cell when library is loaded, including during any washing and reloading steps. The shield can be removed when the library has been removed from the flow cell.

20 Place the light shield onto the flow cell, as follows:

1. Carefully place the leading edge of the light shield against the clip. **Note:** Do not force the light shield underneath the clip.
2. 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.

21 The MinION Flow Cell Light Shield is not secured to the flow cell and careful handling is required after installation.

22 Close the device lid and set up a sequencing run on MinKNOW.

3. Flow cell reuse and returns

1 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°C to +8°C.

The Flow Cell Wash Kit protocol is available on the Nanopore Community.

2. We recommend you to wash the flow cell as soon as possible after you stop the run. However, if this is not possible, leave the flow cell on the device and wash it the next day.

3. Alternatively, follow the returns procedure to send the flow cell back to Oxford Nanopore.

Instructions for returning flow cells can be found here.

4. 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.