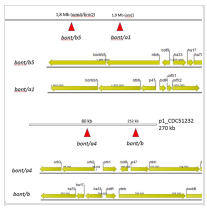

Version 5

Sep 07, 2022

Setting a sequencing run with a nanopore MinION and the Rapid Sequencing gDNA kit (SQK-RAD004) V.5

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ABSTRACT

This protocol is to help in setting up a MinION sequencing run using the rapid sequencing kit from Nanopore (SQK-RAD004). It contains all steps and material need for a successful run. This kit is recommended for rapid preparation time and limited laboratory equipment. The output is lower than that generated by the ligation sequencing kit.

preparation time approximately 15 minutes and require a minimum of 400 ng per reaction.

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EXTERNAL LINK

https://community.nanoporetech.com/

PROTOCOL CITATION

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KEYWORDS

MinION, setting up a NAnopore run, rapid sequencing kit, RAD004, SQK-RAD004

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GUIDELINES

The Rapid Sequencing Kit contains enough reagents to perform six sequencing runs:

Box 1: Contains all reagents for the sequencing step (store at -20C)
Box 2: Contains all the reagents for flowcell priming (store at -20C) - Flush Buffer (FB – blue caps) and Flush tether (FLT - purple cap)

MATERIALS TEXT

Equipment:

- MinION device (MK1B)
- PC with 64 bit Windows 10 or 64 bit Linux (Intel i7, i9, Xeon or better, 16GB RAM, NVIDIA GPU at least 16GB GPU memory, 1T SSD)
- High speed internet high speed or ethernet connection with access to the internet
- Thermocycler
- Microcentrifuge

Supplies:

- SQK-RAD004 kit
- Flowcell (9.4.1 -lower accuracy or 10.4.1 - high accuracy)
- Pipette Tips, sterile, filtered (assorted volumes)
- Microcentrifuge for 0.2 ml and 0.5 ml tubes
- Tubes, 0.2 ml, sterile (Thermofisher cat# AB0337 or equivalent)
- Nuclease-free water (e.g. ThermoFisher, cat # AM9937)
- Timer

BEFORE STARTING

Before using this protocol:

1. **Make sure that you have extracted the DNA of the bacteria or other organism** (if long reads needed, I suggest using a tested DNA extraction kit or in house DNA extraction that renders high quality less sheared DNA).
2. **Quantify the DNA** (I suggest using the Qubit Flourometer for determining the DNA concentration).
3. **Check that the PC to be used for the run is compatible with the process.**
4. **Make sure that the MinKnow Software version** (software installed in the PC to run the MinION) **is up to date (search the community and download the latest version if the version on the PC is not up to date).**
5. **Make sure that you have all supplies (see Materials section).**

Preparation of the DNA Library:

1 Thaw all reagents in box 1 and 2 of the RAD004 kit.

2 Take a flow cell from **4-8 °C** and leave it at room temperature (RT).

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3 Prepare the DNA to a concentration of **400 ng** total in **7.5 µL** , or a concentration of 54 ng/ul in a **0.2 mL** sterile thin-walled PCR tube. Adjust the volume using nuclease-free water. Mix by gently flicking the tube 5 times. DO NOT vortex. Spin down briefly in a micro-centrifuge.

4 Add **2.5 µL** of FRA to the tube containing the prepared DNA from the previous step. Mix by gently flicking the tube as above and spin down.

5 Incubate in Thermocycler at **30 °C** for **00:01:00** , then at **80 °C** for **00:01:00** . 4m
Let it cool at RT for **00:02:00** .

6 Add **1 µL** of RAP to the tube. Mix gently by flicking 5 times and spin down. Incubate for 5m
00:05:00 . After this step your library DNA is ready for mixing with the sequencing reagents and loading to the flow cell. While this step is running you can start setting up the priming of the flow cell.

Flow cell priming:

7 Flow cell priming:

Mix FB and FLT (independently) by vortexing and spin down. Then add **30 µL** of FLT to 1 tube of FB and mix by pipetting thoroughly. This mixture will be use to prime the flow cell.

8 On the PC attached to the MinION, launch the Minknow Software. Place the RT flow cell in the MinION device and proceed with the flow cell QC by clicking on flow cell check (in the Minknow software) and wait till the program reports back how many live pores are in the flow cell. If live pores below 800, do not continue sequencing (if very important samples) . Contact the vendor if the flow cell is still under warranty for a replacement.

9 If the flow cell passed QC then open the priming port by sliding the priming port cover clockwise.

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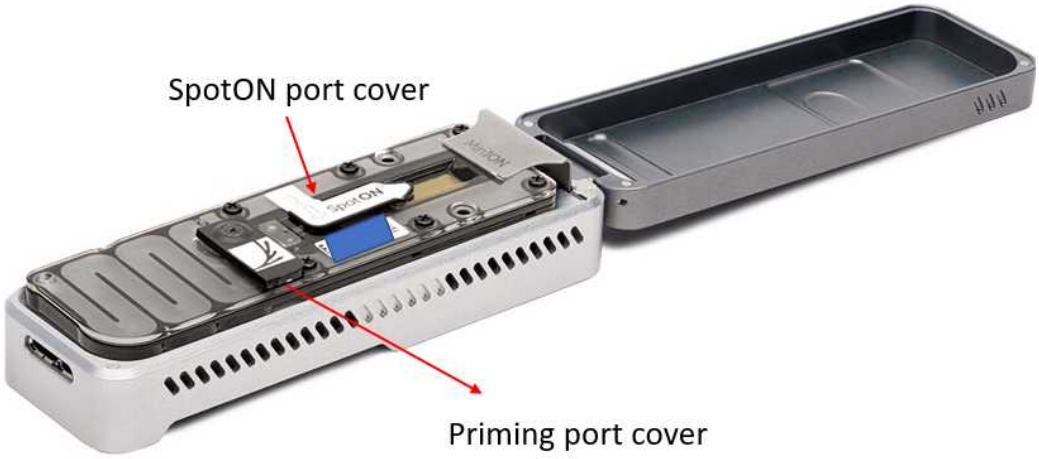

MinION device showing the priming port and the SpotON locations on the flow cell.

9.1 By inserting a pipette tip on the priming port, remove approximately **30 µL** avoiding introducing bubbles.

9.2 Add **800 µL** of the FB+FLT solution prepared on step 7 to the priming port, avoiding introducing bubbles, by slowly dialing down the pipette. Wait **00:05:00** . 5m

9.3 After the **00:05:00** , gently open the flow cell SpotON cover and expose the loading port. Add **200 µL** of FB+FLT to the priming port (NOT the SpotON port). Make sure that the loading port does not have any bubble. 5m

Preparation of the sequencing Library:

10 To the tube containing the DNA library add the following:

- **34 µL** of the sequencing buffer (SQB) - red
- **25.5 µL** of loading beads (LB) - pink (Thoroughly mixed by vortexing before use)
- **4.5 µL** of nuclease-free water

Mix thoroughly by pipetting and make sure that the solution looks homogenous. This is the

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sequencing reaction ready to be loaded to the flow cell.

Loading the flow cell

11 Add the **75 µL** of the sequencing library to the SpotON port in a dropwise fashion. Make sure that every drop falls into the port and that it goes in, before adding the next drop.

Replace the cover over the SpotON and close the priming lid port, and close the MinION device lid

Sequencing 2d

12 On the Minknow software select the flowcell and hit Start, then Start sequencing and follow 2d the instructions for setting the sequencing run. Experiment name (I use the same as the flow cell serial number), then select the kit (RAD004), The run options I leave it mostly default except that I change the time to **48:00:00** , the basecalling to fast (or high accuracy, your choice), Leave the output default C:/Data (or you can point it to wherever you want the run to be save), then just click start at the end of the sequencing setup and your sequencing run will start. By default the output format is fast5 and fastq files, with the fastq file containing 4,000 reads. You can modify the number of reads per fastq if needed.

Check that the flow cell passes the QC again and look for sequencing performance:

Example below. Passed reads closer to the total reads, meaning most reads passed the QC filter, and there has been few failed reads.

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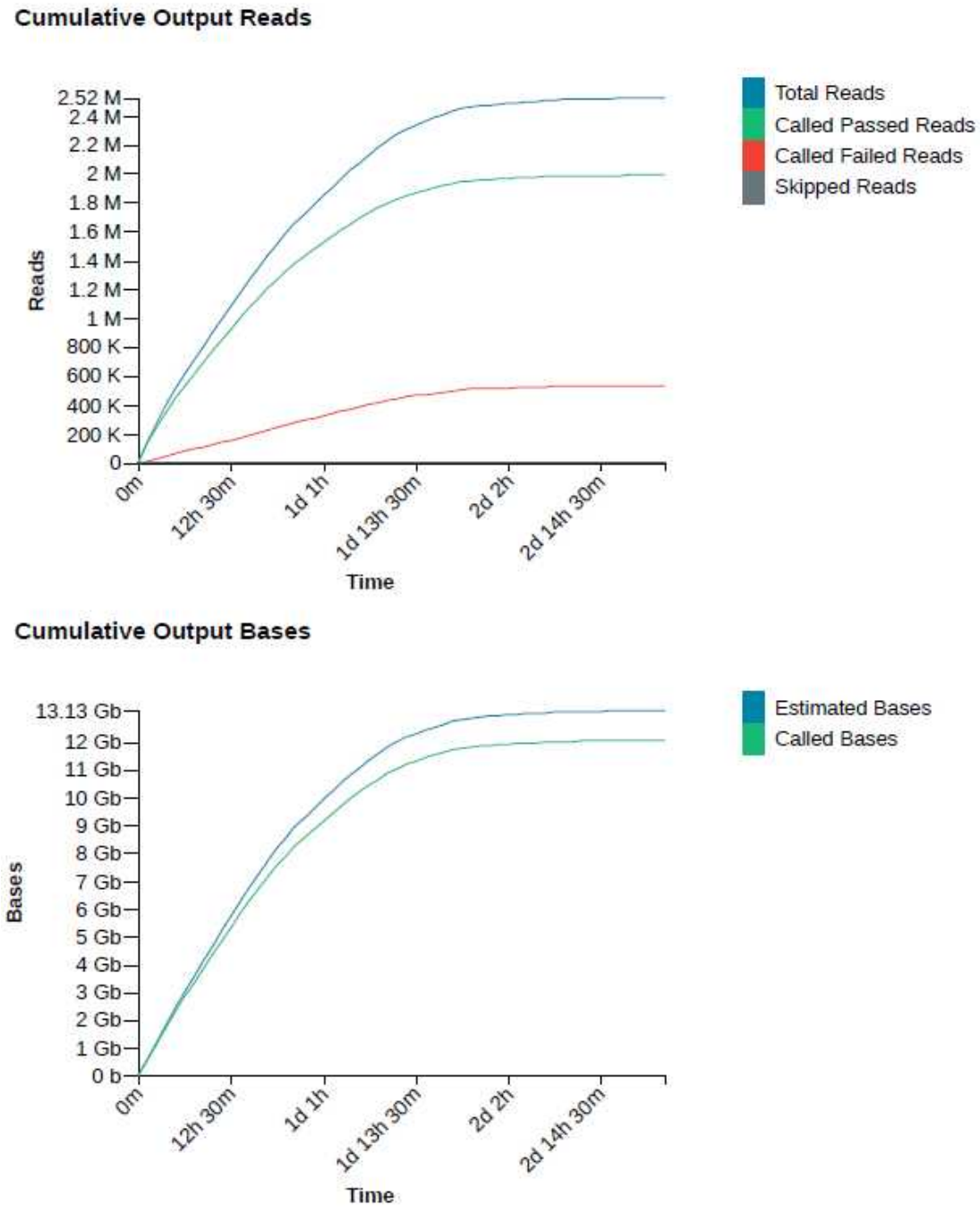

Final output of a successful run.

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