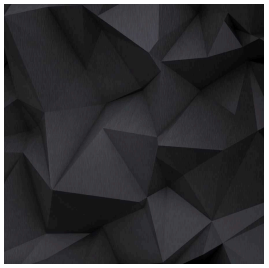
Mar 17, 2023 Version 4

ONT Flongle Flowcell Loading with Q20+ (V14) Chemistry V.4



In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

Overview: This protocol describes the steps used to load a 10.4.1 Flongle flowcell utilizing the Q20+ (V14) Ligation Sequencing Kit from ONT.

This protocol has been tested with Flongle R10.4.1 flowcells.

Time required: 10-15 minutes

Materials

Reagents and Consumables

Ligation Sequencing Kit V14 **Oxford Nanopore Technologies Catalog #**SQK-LSK114

Flongle Sequencing Expansion **Oxford Nanopore Technologies Catalog #**XP-FSE002

100-200uL pipette tips
10uL pipette tips
1.5uL Eppendorf LoBind tubes

Equipment

10uL pipette
100uL pipette
Mini centrifuge
Flongle Starter Pack: $1,460.00
MinION Mk1B Starter Pack: $1000.00

Software

MinKNOW

Protocol materials

Ligation Sequencing Kit V14 **Oxford Nanopore Technologies Catalog #**SQK-LSK114

Flongle Sequencing Expansion **Oxford Nanopore Technologies Catalog #**XP-FSE002

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Troubleshooting

Starting out

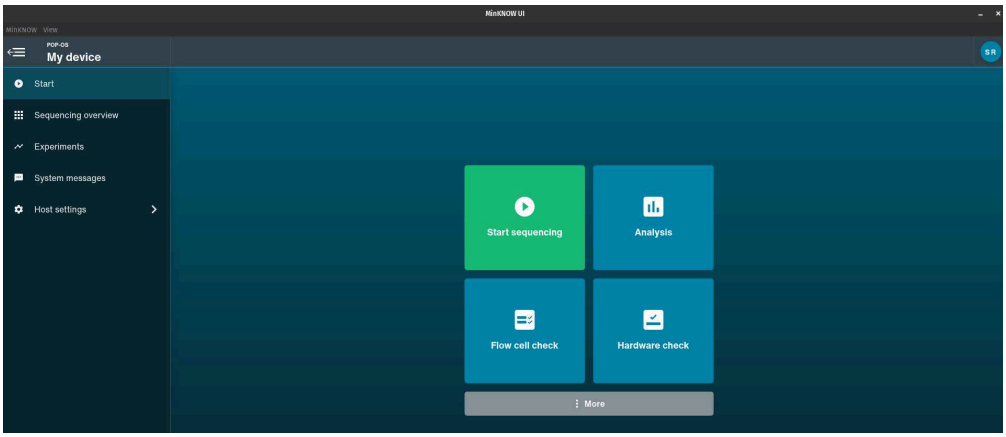
1 Before starting, watch the first 13 minutes of this video: https://vimeo.com/651243660

It covers all of the vital aspects of this protocol.

2 Begin by restarting your computer. This will help to ensure there are no performance issues during your run or other programs running that may conflict with the software.

Attach the USB cable from the computer to the MinION device. Place the Flongle module with the white configuration cell into the MinION device. The top portion of the Flongle should slide underneath the clip and it should gently sit on the top of the device.

Open MinKNOW on the computer and perform a hardware check on the configuration cell. (Start → Hardware Check) The hardware check should pass. You can validate this on the "System Messages" tab.



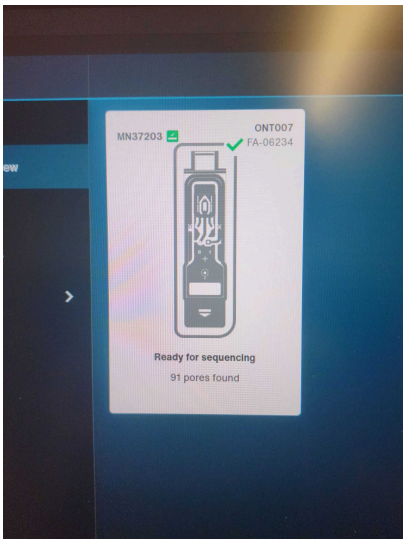
The primary screen within MinKNOW.

3 Wearing gloves, remove a flowcell from the fridge and remove the outer packaging. Remove the configuration cell and place the flowcell into the Flongle device on the MinION. Be sure not to touch any of the electrodes on the back of the cell or the contact pads on the top of the Flongle. It should snap into place with a click. Place the configuration module into the empty pouch from the Flongle until the run is completed. Run a flowcell check within the MinKNOW software. (Start → Flow cell check) There should be at least 50 useable pores.

If the flowcell does not pass the QC test, remove it and repeat with a new cell. ONT will do a warranty replacement on this cell. If a cell is burned, I will still use it after I get a full

run. Just put it back in the wrapper and put it in the fridge. Once the good run is complete, I will run that cell with the same library we just created to get some free extra data for the run.

My first flowcell test had 87 useable pores. Future tests up to 6 weeks after receipt still passed with 70+ useable pores.



The Sequencing Overview screen should show the number of pores that are active and ready for sequencing.

Prepare the loading solution

4 Thaw the Sequencing Buffer (SB), Library Beads (LIB), Flow Cell Flush (FCF), and Flow Cell Tether (FCT) at room temperature.

Flow Cell Tether (FCT) is found in the

Ligation Sequencing Kit V14 **Oxford Nanopore Technologies Catalog #**SQK-LSK114

All of the remainder are in the

Flongle Sequencing Expansion **Oxford Nanopore Technologies Catalog #**XP-FSE002

Original full V14 Ligation Sequencing Kit (Q20+) protocol can be found here:

FILE ligation-sequencing-amplicons-sqk...

5 Mix the Sequencing Buffer (SB), Flow Cell Tether (FCT) and Flow Cell Tether (FCT) tubes by vortexing and spin down at room temperature.

6 In a 1.5uL tube, mix 117uL of the Flow Cell Flush (FCF) with 3uL of the Flow Cell Tether (FCT) and mix by pipetting.

7 Peel back the seal tab from the Flongle flow cell to the point where the sample port is exposed and hold the seal tab open by using the adhesive on the tab to stick it to the lid of the MinION.

Video of the process: https://youtu.be/zlkTfRf8g7I (2 minutes)

8 Using a 200uL pipette, bring up the FB/FLT mix into the tip. This is your priming solution.

VERY IMPORTANT: there should be NO air bubble in the tip of the pipette. Introducing any air bubbles will destroy any nanopores that the air bubble contacts.

If there is any air in the tip, turn the dial of the pipette clockwise (towards 0) to move the air bubble out of the tip. I will typically turn the dial until there is a small amount of fluid hanging as a drop on the exterior of the tip. Then touch that drop to the sample port, bringing the tip down to contact with the port.

Also note, this is the only part of any protocol that I use a high quality 200uL pipette tip for. Every other protocol/step, I use unfiltered bulk tips that are sterilized in a pressure cooker. For this step, I use prepackaged and higher quality tips for. There have been a couple instances where the fluid built up at the port opening of the flowcell, rather than being injected into the flowcell, and I believe it was due to the quality of the tips being used.

9 Place the tip of the pipette securely into the sample port. Turn the dial of the pipette clockwise a time or two and you should see a small amount of yellow-green fluid come into the tip. This helps to ensure there are no air bubbles present.

Once confirmed, slowly dispense the priming fluid into the flowcell by slowly turning the dial of the pipette clockwise (towards 0). Do not push down on the plunger to eject the fluid.

You should be able to see the storage liquid entering the waste port as you enter it into the flowcell.

STOP LOADING just before the liquid in the tip reaches the bottom. You do not want to push any air into the flowcell

Prepare Library

10 In a new 1.5uL tube, add the reagents below in the order they are listed.

Note: The LIB settles quickly, so vortex it thoroughly before removing it from the tube.

Reagent	Volume
Sequencing Buffer (SB)	15uL
Library Beads (LIB)	10uL
3 - 20 fMol of the DNA library	5uL
Total	*30uL*

Mix the solution by gently pipetting up and down 10-20 times.

11 Bring the entire volume into the tip of a pipette.

VERY IMPORTANT: Once again make sure there are no air gaps. Bring a small drop of fluid outside of the tip.

Insert the tip of the pipette into the sample port and slowly dispense the library into the flowcell by slowly turning the dial of the pipette clockwise (towards 0). Do not push down on the plunger to eject the fluid.

STOP LOADING before the liquid in the tip reaches the bottom. You do not want to push any air into the flowcell

12 Seal the Flongle cell be folding the tab back down, using the adhesive on the seal tab. Ensure that the wheel section covers the loading port and the two dots cover each waste port. Gently press over the tab to ensure a good seal. (Do not do a wiping press, just an up-and-down press.)

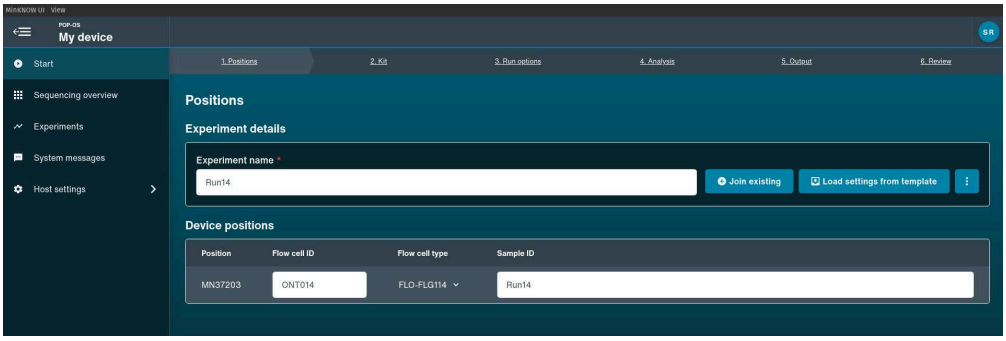
13 Close the MinION lid and start your sequencing run.

Start → Start Sequencing

Run Name: Ex - Run16 Flow cell type: FLO-FLG114

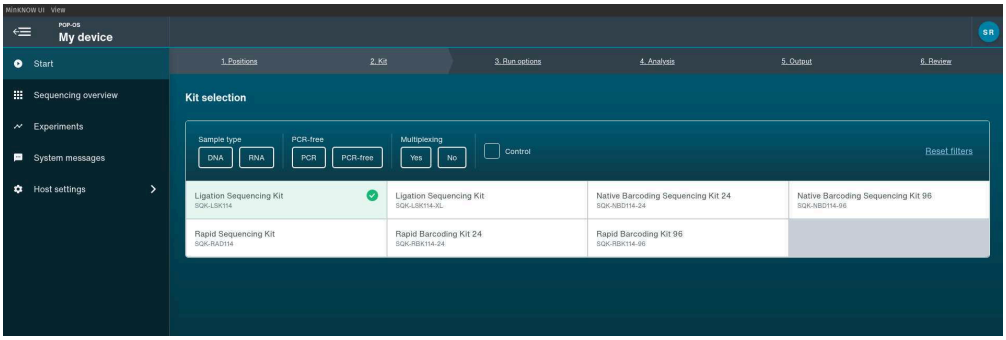
Note: If this flow cell is not an option, you may need to update your version of MinKnow.

Step 1: Positions
Flow cell ID: Ex - Run16 Sample ID: Ex - Run16 Continue to kit selection >



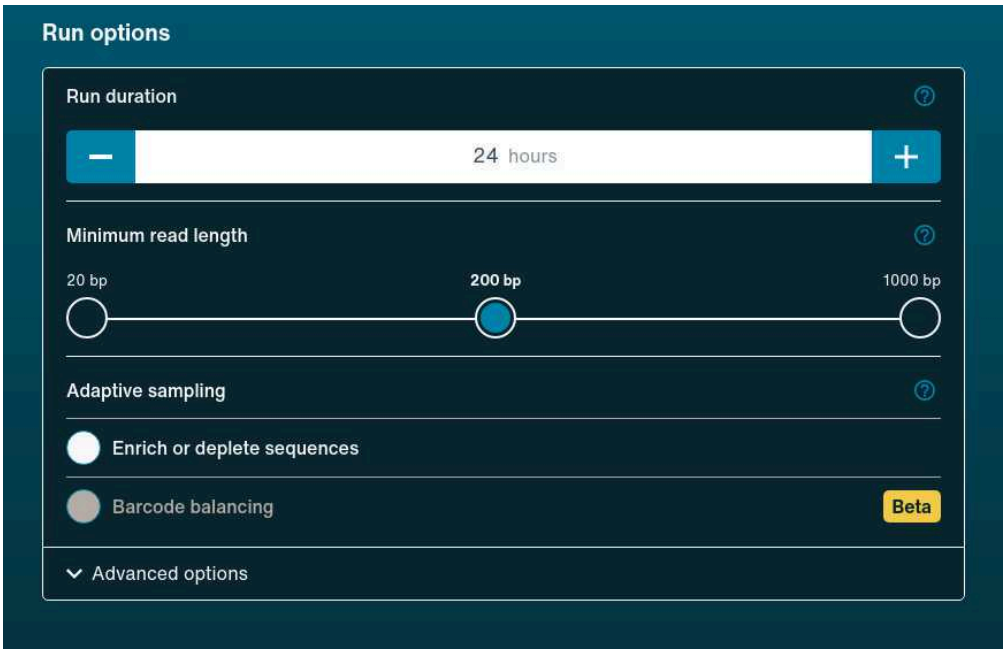
Step 1: Positions

Step 2: Kit
Sample Type: DNA PCR-free: PCR Multiplexing: Yes
These three options just filter the kits to select from.
Select: Ligation Sequencing Kit (LSK-114) Continue to run options >



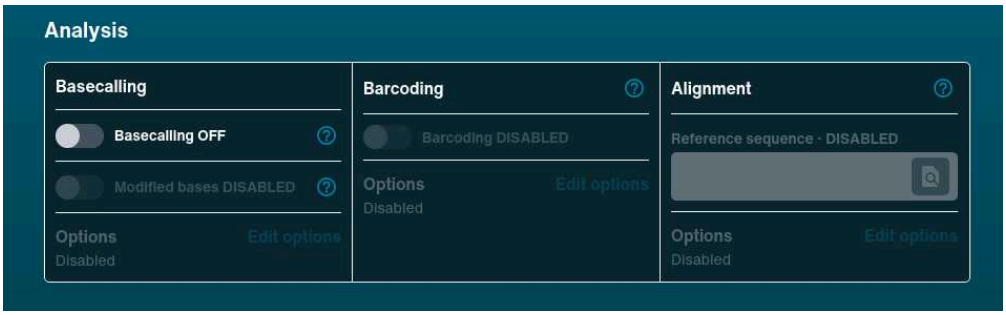
Step 2: Kit

Step 3: Run options
(All should be at defaults)
Run Duration: 24 hours
Minimun read length: 200 base pairs
Active Channel Selection: On
Time between pore scans: 1.5 hours
Reserve pores: off
Continue to analysis >



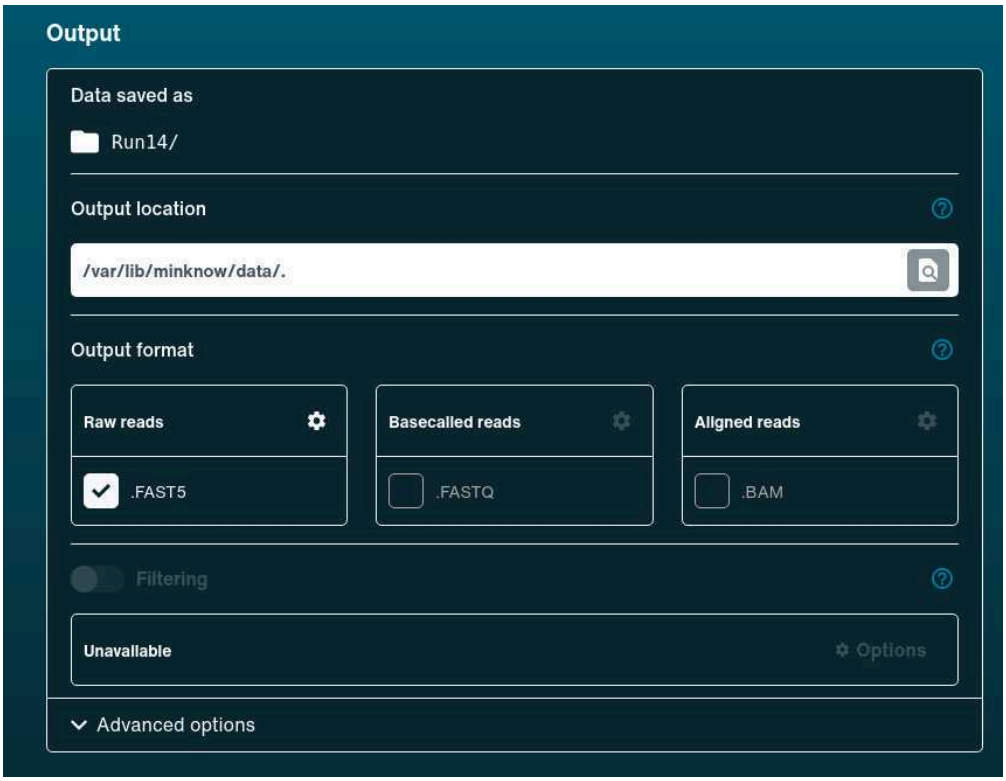
Step 3: Run Options

Step 4: Analysis
Basecalling: Off



Step 4: Analysis

Step 5: Output
(All should be defaults)
Output location: /var/lib/minknow/data/.
Output format: fast5
Output bulk file: on
Continue >



Step 5: Output

On the final step you can save this as a template for future runs.