

VERSION 4

MAR 16, 2023

OPEN ACCESS

**DOI:**  
[dx.doi.org/10.17504/protocols.io.ewov1nm5pgr2/v4](https://dx.doi.org/10.17504/protocols.io.ewov1nm5pgr2/v4)

**Protocol Citation:** Stephen Douglas Russell 2023. ONT Flongle Flowcell Loading with Q20+ (V14) Chemistry. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.ewov1nm5pgr2/v4> Version created by Stephen Douglas Russell

**License:** This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

**Created:** Mar 14, 2023

**Last Modified:** Mar 16, 2023

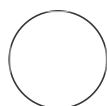
**PROTOCOL integer ID:**  
 78716

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

Stephen Douglas Russell<sup>1</sup>

<sup>1</sup>The Hoosier Mushroom Society

The Hoosier Mushroom Society



Stephen Douglas Russell

### 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

# Starting out

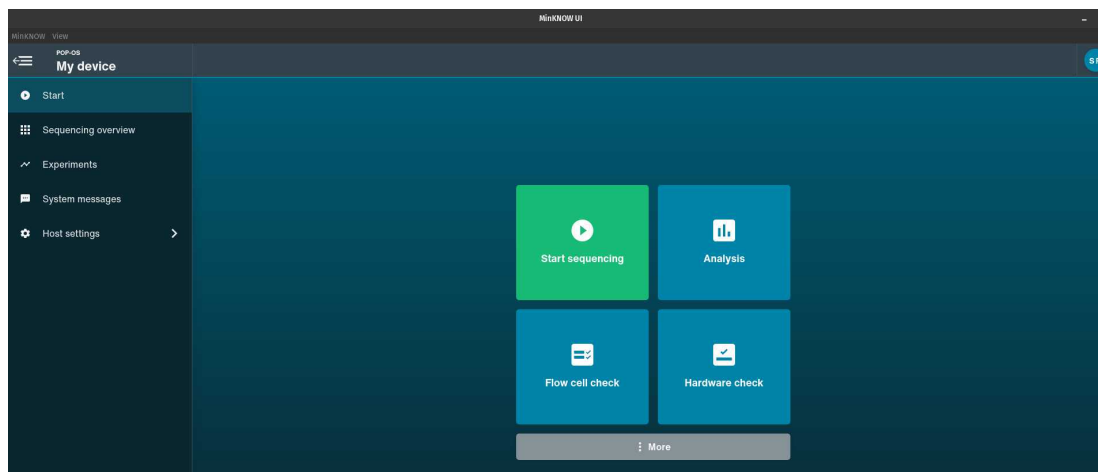
- 1 Before starting, watch the first 13 minutes of this video: <https://vimeo.com/651243660>

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.



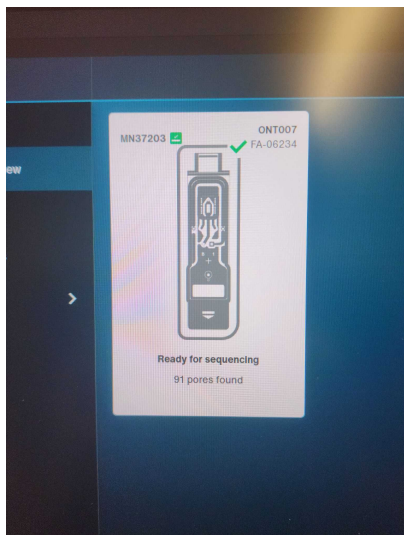
The primary screen within MinKNOW.

- 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:

 [ligation-sequencing-amplicons-sqk-lsk114-ACDE\\_9163\\_v114\\_revJ\\_29Jun2022-flongle.pdf](https://dx.doi.org/10.17504/protocols.io.ewov1nm5pgr2/v4)

- 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
<i>Total</i>	<i>30uL</i>

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 by 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 >

MinION v1.10.0 View

My device

Start

Sequencing overview

Experiments

System messages

Host settings

1. Positions

2. Kit

3. Run options

4. Analysis

5. Output

6. Review

Positions

Experiment details

Experiment name \*

Run14

Join existing

Load settings from template

Device positions

Position	Flow cell ID	Flow cell type	Sample ID
MN37203	ONT014	FLO-FLG114	Run14

## 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 >

MinION v1.10.0 View

My device

Start

Sequencing overview

Experiments

System messages

Host settings

1. Positions

2. Kit

3. Run options

4. Analysis

5. Output

6. Review

Kit selection

Sample type

DNA RNA

PCR-free

PCR PCR-free

Multiplexing

Yes No

Control

Reset filters

Ligation Sequencing Kit SQK-LSK114	Ligation Sequencing Kit SQK-LSK114-XL	Native Barcoding Sequencing Kit 24 SQK-NBD114-24	Native Barcoding Sequencing Kit 96 SQK-NBD114-96
Rapid Sequencing Kit SQK-RAD114	Rapid Barcoding Kit 24 SQK-RBK114-24	Rapid Barcoding Kit 96 SQK-RBK114-96	

## Step 2: Kit

## Step 3: Run options

(All should be at defaults)

Run Duration: 24 hours

Minimum read length: 200 base pairs

Active Channel Selection: On

Time between pore scans: 1.5 hours

Reserve pores: off

Continue to analysis >

### Run options

Run duration ?

—

24 hours

+

---

Minimum read length ?

20 bp
200 bp
1000 bp

---

Adaptive sampling ?

☐ Enrich or deplete sequences
☐ Barcode balancing

Beta

---

▼ Advanced options

### Step 3: Run Options

### Step 4: Analysis

Basecalling: Off

### Analysis

Basecalling	Barcoding	Alignment
<input type="checkbox"/> <b>Basecalling OFF</b> <span>?</span>	<input type="checkbox"/> <b>Barcoding DISABLED</b> <span>?</span>	<input type="checkbox"/> <b>Reference sequence - DISABLED</b> <span>?</span>
<input type="checkbox"/> <b>Modified bases DISABLED</b> <span>?</span>	<div>Options <span>Edit options</span></div> Disabled	<div>Options <span>Edit options</span></div> Disabled
<div>Options <span>Edit options</span></div> Disabled		<div>Options <span>Edit options</span></div> Disabled

### Step 4: Analysis

### Step 5: Output

(All should be defaults)

Output location: /var/lib/minknow/data/.

Output format: fast5

Output bulk file: on

Continue >

**Output**

Data saved as  
Run14/

Output location  
/var/lib/minknow/data/

Output format

Raw reads	Basecalled reads	Aligned reads
<input checked="" type="checkbox"/> .FAST5	<input type="checkbox"/> .FASTQ	<input type="checkbox"/> .BAM

☐ Filtering

Unavailable Options

Advanced options

## Step 5: Output

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