Priming and loading a MinION flowcell v2

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Aug 25, 2020

1 Works for me This protocol is published without a DOI.

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EXTERNAL LINK
https://doi.org/10.1016/j.remle.2020.05.007

PROTOCOL CITATION
Josh Quick 2020. Priming and loading a MinION flowcell v2. **protocols.io** https://protocols.io/view/priming-and-loading-a-minion-flowcell-v2-bkatksen

EXTERNAL LINK
https://doi.org/10.1016/j.remle.2020.05.007

CREATED
Aug 25, 2020

LAST MODIFIED
Aug 25, 2020

PROTOCOL INTEGER ID
41011

PARENT PROTOCOLS
In steps of
nCoV-2019 sequencing protocol v3 (LoCost)

1 Thaw the following reagents at room temperature before placing on ice:

Sequencing buffer (SQB)
Loading beads (LB)
Flush buffer (FLB)
Flush tether (FLT)

2 Add **30 µl** FLT to the FLB tube and mix well by vortexing.

3 If required place a new MinION flowcell onto the MinION by flipping open the lip and pushing one end of the flowcell under the clip and pushing down gently.

4 Rotate the inlet port cover clockwise by 90° so that the priming port is visible.

5 Take a P1000 pipette and tip and set the volume to **800 µl** . Place the tip in the inlet port and holding perpendicularly to the plane of the flowell remove any air from the inlet port by turning the volume dial anti-clockwise.

Be careful not to remove so much volume that air is introduced onto the rectangular array via the outlet.

6 Load **800 µl** of FLB (plus FLT) into the flow cell via the inlet port, dispense slowly and smoothly trying to avoid the introduction of any air bubbles.

7 Wait for **00:05:00** .

8 Gently lift the SpotON cover to open the SpotON port.

9 Load another **200 µl** of FLB (plus FLT) into the flow cell via the inlet port, this will initiate a siphon at the SpotON port to allow you to load the library dilution.

10 In a new tube prepare the library dilution for sequencing:

Component	Volume
SQB	37.5 µL
LB	25.5 µL
Library	12 µL
Total	**75 µL**

Mix LB immediately before use as they settle quickly.

Make up with EB if less than 12 µL library is required.

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

12 Add the **75 µl** library dilution to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop siphons into the port before adding the next.

13 Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the inlet port and close the MinION lid.