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Priming and loading a MinION flowcell

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

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Troubleshooting

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.

> Note
>
> 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
Final library	12 µL
Total	75 µL

Note

Mix LB immediately before use as they settle quickly.

Dilute library in EB if 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.