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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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- 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 flowcell 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.