SOP

10.3.6 Oxford Nanopore Sequencing – priming and loading a flow cell

Objectives and scope

This SOP describes the various steps to follow to carry out priming and loading of a flow cell (the SpotON flow cell) for sequencing purposes using the nanopore method.

This SOP is intended for Mini-Lab laboratory technicians.

Principle

During this procedure the DNA library previously prepared (SOP 10.3.5) is loaded onto the Nanopore Flow Cells for sequencing.

Note 1: The flow cell needs to be primed before loading the DNA into it, i.e. check that the flow cell has enough pores for a good sequencing run.

Safety and environment

- Wear your PPE for the duration of this technique: lab coat, gloves;
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

Flow cells are irreversibly damaged when frozen!

All flow cells should be stored at 2-8°C from receipt to ensure optimal performance.

Sample

- Type of material:
  - DNA libraries prepared from gDNA (SOP 10.3.5)

Equipment

Common Name	*Associated SOP*
Freezer -20°C	SOP-7.7-REFCON
SpotON Flow Cell MIN114 R10	SOP TBD

Commenté [1]: to be done

Consumables

*Common Name**	*Storage conditions*
Nuclease-free water	*2-25°*
Gloves	*N/A*
1.5 ml Eppendorf DNA LoBind tubes	*N/A*
P1000 pipette and tips	*N/A*
P100 pipette and tips	*N/A*
P10 pipette and tips	*N/A*

Rapid Barcoding Kit 96 (SQK-RBK114.96) materials

Name	*Acronym*	*Cap colour*	*N of vials*	*Fill volume per vial (µL)*	*Storage conditions*
Sequencing Buffer II	**SBII**	*Red*	1	500	
Loading Solution	**LS**	*White cap, pink label*	1	400	
Loading Beads II	**LBII**	*Pink*	1	360	
Flush Tether	**FLT**	*Purple*	1	400	*N/A*
Flush Buffer	**FB**	*White*	1 bottle	1500	

Flush Tether (FLT)	30 µl
Flush Buffer (FB)	1.170 µl

Priming and Loading a Flow Cell

Before starting

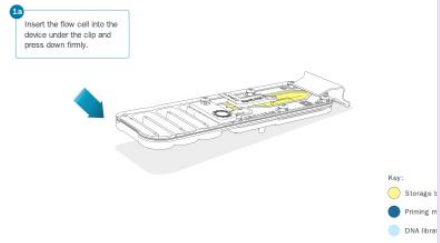
Thaw the Sequencing BufferII (SBII), Loading Beads II (LBII) or Loading Solution (LS, if using), Flush Tether (FLT) and one tube of Flush Buffer (FB) at room temperature before mixing the reagents by vortexing and spin down at room temperature.

1. Prepare the flow cell priming mix as in the table below in a suitable vial for the number of flow cells to flush (multiply the volume by the number of flow cells). Once combined, mix well by briefly vortexing.

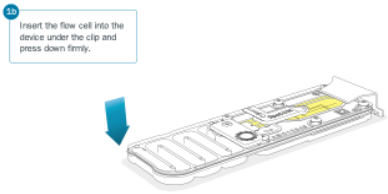
Priming Mix	
Reagent	Volume per flow cell

2. Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact.
   a. *Insert the flow cell into the device under the clip. Press down firmly on the flow cell to ensure correct thermal and electrical contact*

Commenté [2]: ONT advice to use 5 uL Bovine Serum Albumine in the priming mix
(Optional) Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, cat# AM2616)



b. Insert the flow cell into the device under the clip and press down firmly



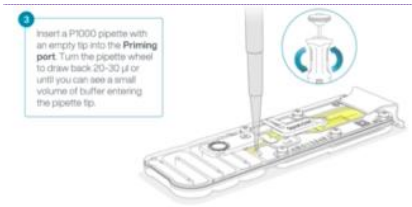
3. Slide the flow cell priming port cover clockwise to open the priming port.

4. After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:
   a. Insert a P1000 pipette with an empty tip into the PRIMING PORT.
   b. Turn the pipette wheel to draw back 20-30 µl or until you can see a small volume of buffer entering the tip

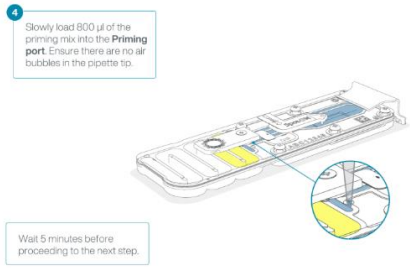
   Note: Visually check that there is continuous buffer (yellow) from the priming port across the sensor array

IMPORTANT:

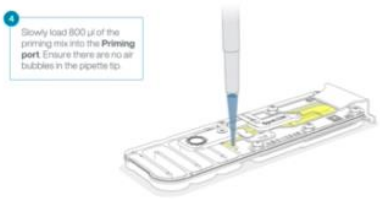
Take care when drawing back buffer from the flow cell. Do not remove more than 20-30µl, and make sure that the array of pores **are covered by buffer at all times**. Introducing air bubbles into the array can irreversibly damage pores!



5. Load 800µl of the priming mix into the flow cell via the priming port, avoiding the introduction of air bubbles.



6. Wait for five minutes. During this time, prepare the library for loading by following the steps below.



Commenté [3]: update numbers

Prepare the library for loading

7. Thoroughly mix the contents of the Loading Beads (LBII) by pipetting.

IMPORTANT:

The Loading Beads II (LBII) tube contains a suspension of beads. These beads settle very quickly. Itis vital that they are mixed immediately before use.

8. In a new tube, prepare the library for loading as follows:

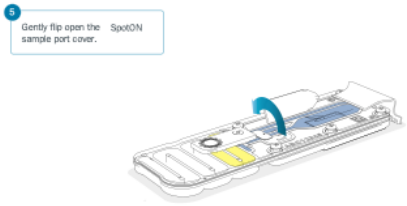
Library Mix	
Reagent	Volume per flow cell
Sequencing Buffer II (SBII)	37.5 µl
Loading Beads II (LBII) mixed immediately before use (or LS if using)	25.5 µl
DNA library	12 µl
Total	**75 µl**

Note1: the use of the LBII is recommended, however, if you have previously used water to load your library, you must use Loading Solution (LS) instead of LBII (?).

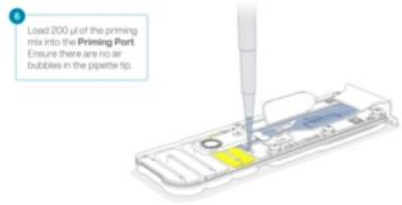
Note2: Load the library onto the flow cell immediately after adding the Sequencing Buffer II (SBII) and Loading Beads II (LBII).

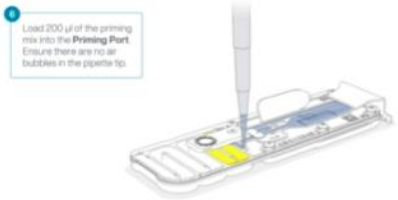
9. Complete the flow cell priming:

   a. Gently lift the SpotON sample port cover to make the SpotON sample port accessible.



   b. Load 200 µl of the priming mix into the flow cell priming port (not the SpotON sample port), avoiding the introduction of air bubbles.





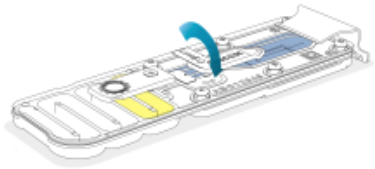
Loading the library on the flow cell

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

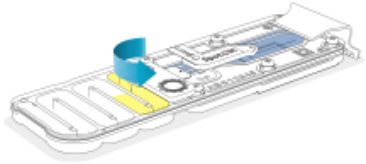
11. Add 75µl of the prepared library to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop flows into the port before adding the next.

Commenté [4]: sequencing buffer = SB
Library beads = LIB,
if your library is more viscous you can use Library Solution (LIS)

12. Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port,



13. Gently close the priming port and replace the MinION or GridION device lid.



Data acquisition and basecalling

How to start sequencing

Once you have loaded your flow cell, the sequencing run can be started on MinKNOW, the sequencing software that controls the device, data acquisition and real-time basecalling.

MinKNOW can be used and set up to sequence in multiple ways:

- On a computer either directly or remotely connected to a sequencing device.
- Directly on a GridION, MinION Mk1C or PromethION 24/48 sequencing device.

To start a sequencing run on MinKNOW:

1. Navigate to the start page and click Start sequencing.
2. Fill in your experiment details, such as name and flow cell position and sample ID.
3. Select the sequencing kit used in the library preparation on the Kit page.
4. Configure the sequencing and output parameters for your sequencing run or keep to the default settings on the Run configuration tab.

Run configuration tab:

Sequencing and analysis

-> basecalling Super Accurate

-> Barcoding/Trim on

Data target-> run limit:

-> choose when the MinIon has to stop sequencing

Commenté [5]: I guess this is on the procedure for dat analysis?

Commenté [6]: this is the right procedure,

Commenté [7]: You can sequence for as many hours as desired or you can let the flowcell run till end of live. We use this last options when we run 48 or 96 samples and want to have as many coverage as possible

Related documents

- Priming video: https://community.nanoporetech.com/nanopore_learning/lessons/priming-and-loading-your-flow-cell
- SOP-10.3.3. DNA extraction for sequencing
- SOP 10.3.4. DNA quantification using Qubit
- SOP-10.3.5: Rapid Sequencing gDNA barcoding and library preparation
- DOC-6.8-DECHINT: 6.8 Internal waste management
- Commercial SOP: Rapid sequencing gDNA -barcoding (SQK-RBK110.96)