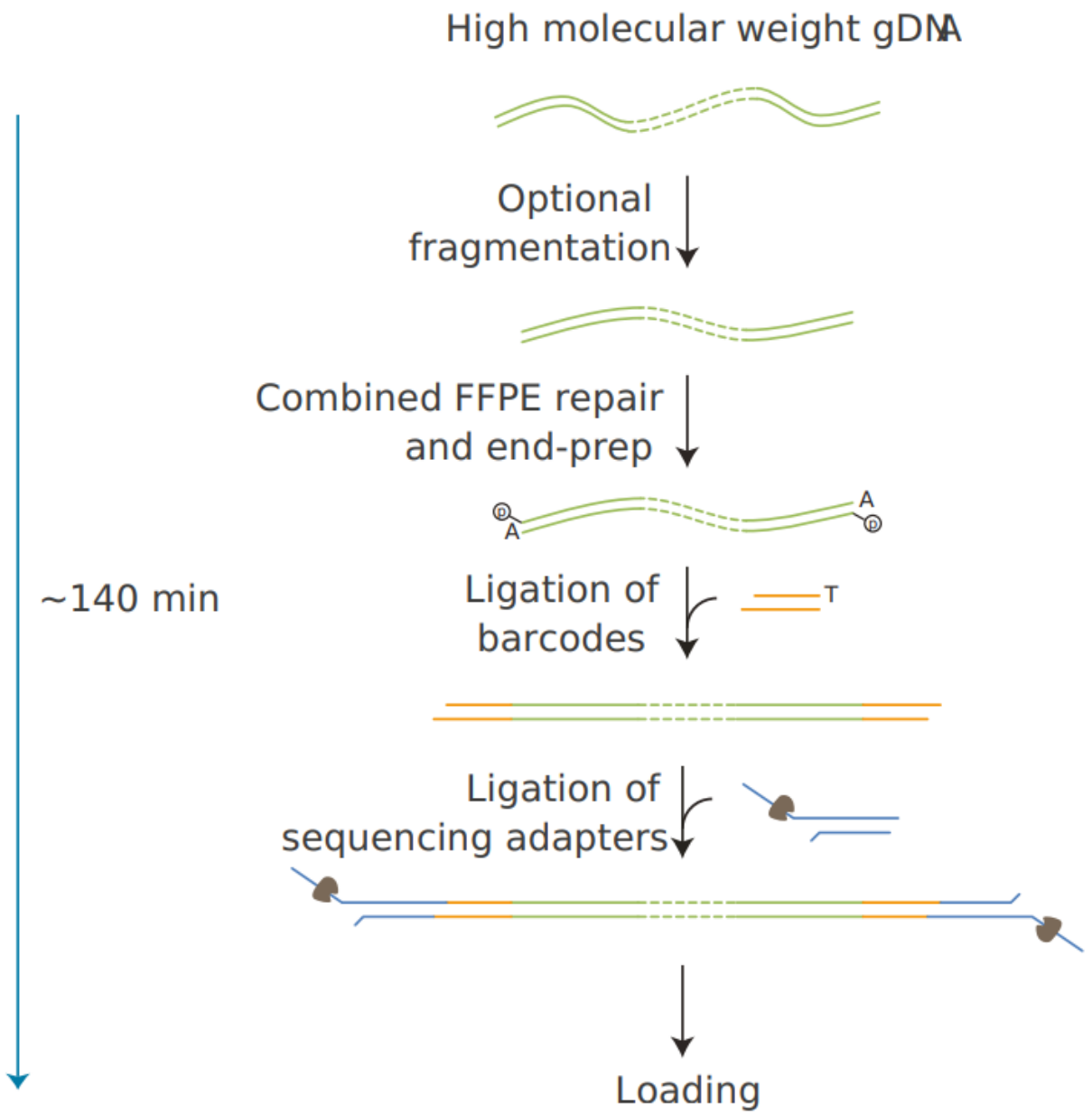
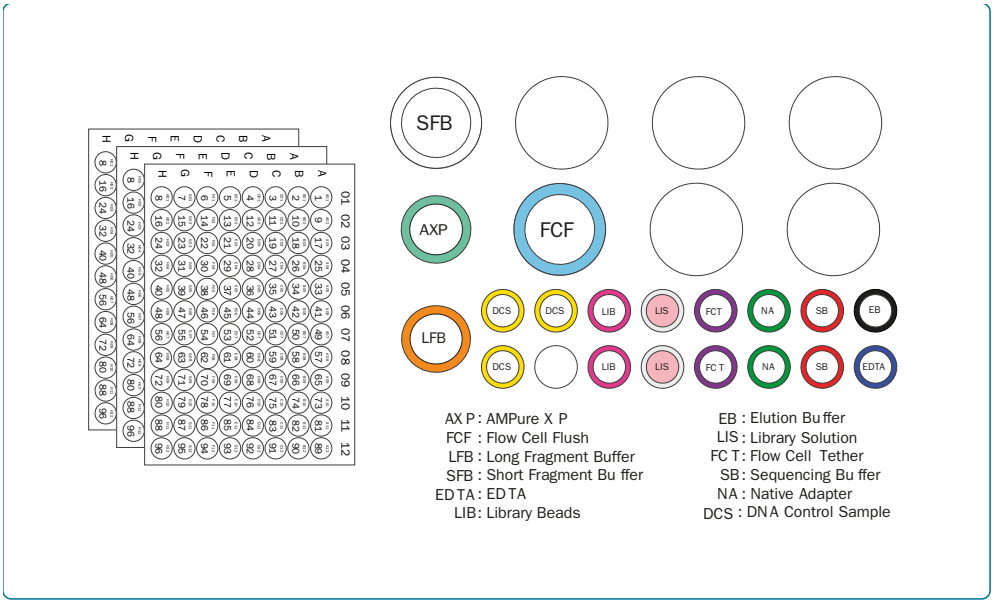
KAPA EvoPrep_SQK-NBD114.96

(0.5X reagent, PromethION)



SQK-NBD114.96 kit



Name	Acronym	Cap colour	No. of vials	Fill volume (µl)
Native Barcode plate	**NB01-96**	**-**	**3 plates**	**8 per well**
DNA Control Sample	DCS	Yellow	3	35
Native Adapter	**NA**	**Green**	**2**	**40**
Sequencing Buffer	SB	Red	2	700
Library Beads	LIB	Pink	2	600
Library Solution	LIS	White cap, pink label	2	600
Elution Buffer	EB	Black	1	1,500
AMPure XP Beads	AXP	Clear cap, light teal	1	6,000
Long Fragment Buffer	LFB	Clear cap, orange label	1	7,500
Short Fragment Buffer	SFB	Clear	1	25,000
EDTA	EDTA	Blue	1	700
Flow Cell Flush	FCF	Clear cap, light blue label	1	15,500
Flow Cell Tether	FCT	Purple	2	200

Source: ONT SQK-NBD114.96 PromethION protocol NBE_9171_v114_revU_15Jul2026, pp. 9-10.
Barcodes run in columns on the plate (A1-H1 = NB01-NB08, A2-H2 = NB09-NB16, ...).

KAPA EvoPrep kits

The KAPA EvoPrep Kits contain:

Kit Material Number	Description	Volume
10154039001 10153806001*	**KAPA EvoPrep Kit (24 rxn)**	
	KAPA End Repair and A-Tailing ReadyMix	600 µL
	KAPA Ligation ReadyMix	240 µL
	KAPA HiFi HotStart ReadyMix (2X)	600 µL

Compatibility of this protocol

This protocol should only be used in combination with:

1. KAPA EvoPrep kit
2. Ligation Sequencing Kit (**SQK-NBD114.96**)
3. R10.4.1 PromethION Flow Cells (**FLO-PRO114M**)

Materials :

1. DNA for every sample to be barcoded for R10.4.1 flow cells
2. KAPA EvoPrep kit
3. Ligation Sequencing Kit (**SQK-NBD114.96**)

Consumables :

1. 1.5 ml Eppendorf DNA LoBind tubes
2. 0.2 ml thin-walled PCR tubes
3. Fresh 80% ethanol in Nuclease-free water
4. Nuclease-free water
5. **PromethION Flow Cell** + Flow Cell Light Shield
6. HyperPure beads (KAPA)

Equipment :

1. Hula mixer (Rotor mixer)
2. **PromethION device**
3. PCR machine
4. magnetic stand
5. Quantus fluorometer
6. Microfuge
7. Vortex mixer
8. Ice bucket with ice
9. Timer
10. Pipette and tips

8個樣本長度約10Kbp 每個DNA取 ~1236ng (~200 fmol) for every sample to be barcoded for R10.4.1 flow cells
每個樣品樣本量參考表 KAPA EvoPrep kit)

Average of DNA length (bp)	Recommended sample concentration (fmol)	Recommended sample quantity (ng)
25,000	100	1,545
20,000	100	1,236
15,000	100	927
10,000	**200**	**1300**
5,000	100	309
2,000	100	124

Step A. End-prep & A-Tailing **(0.5X)**

※ Prewarm : a. PCR machine
b. KAPA EvoPrep kit on ice before use
c. End Repair & A-Tailing Buffer

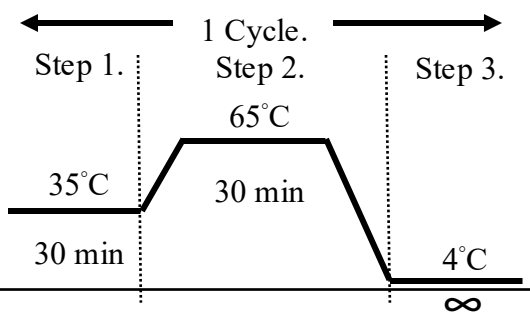
1. Make up each sample to **17.5 µl** using nuclease-free water. Mix gently by pipetting and spin down

3. Combine the following components per tube/well:

Component	KAPA EvoPrep kit
DNA sample	**17.5 µl (200 fmol)**
KAPA ERAT ReadyMix	**12.5 µl**
Total volume	**30 µl**
(sample ≥ ~71 ng/µl at 10 kb; concentrate if needed)	

4. Ensure the components are thoroughly mixed and spin down . Return the plate/tube(s) to ice. Proceed immediately to the next step.

5. PCR program : (Optimal temperature of lid is about 80˚C to 85˚C)



Step B. Barcode Ligation (0.5X)

※ **Prewarm : SQK-NBD114.96 kit**
Native Barcode plate (NB01-96) **– each well is single use**

1. In clean 0.2 ml PCR-tubes or a 96-well plate, add the reagents in the following order per well :
(ONE sample for ONE barcode)

Component (for 1 sample / barcode)	**Volume**
End-preped DNA	**30 µl**
Native Barcode (**NB01-96**)	**1.25 µl**
KAPA Ligation ReadyMix	**5 µl**
Nuclease-Free water	**1.25 µl**
Total volume	**37.5 µl**

2. Mix thoroughly by gently pipetting the reaction up and down several times.

3. Incubate at 20˚C for 5 mins

Step C. Barcode ligation product clean-up (0.5X)
※ **Prewarm : KAPA HyperPure beads resuspend by vortexing before use**
1. Add HyperPure Beads to the pooled reaction, and mix by pipetting for a 1.0X clean (37.5 µl beads per 37.5 µl of pooled reaction; e.g. 300 µl for 8 samples).
2. Incubate on a Hula mixer (rotator mixer) for 10-20 minutes at RT.
3. **Prepare 10 mL of fresh 80% ethanol in Nuclease-free water**.
4. Spin down the sample and pellet on a magnetic stand **for 5 minutes**
5. Keep the tube on the magnetic stand **until the eluate is clear and colourless, and pipette off the supernatant.** and **pipette off the supernatant**
6. Keep the tube on the magnetic stand and wash the beads with **700 µl of freshly- prepared 80% ethanol** without disturbing the pellet.
7. Remove the ethanol using a pipette and discard.
8. Repeat the previous step to remove ethanol.
9. Spin down and place the tube back on the magnetic stand.
10. **Pipette off any residual ethanol**
11. Allow to dry for ~30 seconds, but do not dry the pellet to the point of cracking
12. Remove the tube from the magnetic stand and resuspend the pellet in **35 µl Nuclease-free water**.
13. **Incubate for 10-20 minutes at 37˚C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution.**
14. Pellet the beads on a magnetic stand until the eluate is clear and colourless
15. **Remove and retain 35 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.**
16. Quantify 1 µl of eluted sample **using a Quantus fluorometer (or Qubit).**

HyperPure beads for the 1.0X clean-up of the pooled reaction (37.5 µl per sample):

Samples (N)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Beads (µl)	37.5	75	112.5	150	187.5	225	262.5	300	337.5	375
Samples (N)	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Beads (µl)	412.5	450	487.5	525	562.5	600	637.5	675	712.5	750

Step D. Adapter Ligation and clean-up	
※ **Prewarm : SQK-NBD114.96 kit** Native Adapter (NA)	
1. In a 1.5 ml Eppendorf LoBind tube, mix in the following order:	
Component (Mix all samples)	**Volume**
Pool ALL barcoded sample	**30 µl** (pooled eluate from Step C)
Native Adapter (NA)	**2.5 µl**
KAPA Ligation ReadyMix	**5 µl**
Total volume	**37.5 µl**
2. Mix thoroughly by gently pipetting the reaction up and down several times.	
3. Incubate at 20˚C for 5 mins	

Step E. DNA library clean-up

※ **Prewarm : KAPA EvoPrep kit**
a. HyperPure beads
SQK-NBD114.96 kit at RT before use
b. Long Fragment Buffer (LFB)
c. Short Fragment Buffer (SFB)
d. Elution Buffer (EB)

1. Add **37.5 µl (1X) of resuspended HyperPure beads** to the **37.5 µl adapter-ligated reaction** and mix by pipetting.
2. Incubate on a Hula mixer (rotator mixer) for 20 minutes at RT.
3. Place on magnetic stand, allow beads to pellet and pipette off supernatant.
4. Wash the beads by adding either **125 µl Long Fragment Buffer (LFB) or 125 µl Short Fragment Buffer (SFB)(<3k).**
※ **Do not use ethanol to wash the beads !**
5. Flick the beads to resuspend, then return the tube to the magnetic stand and allow the beads to pellet.
6. Remove the supernatant using a pipette and discard.
7. **Wash the beads** by adding either **125 µl Long Fragment Buffer (LFB) or 125 µl Short Fragment Buffer (SFB)(<3k).**
※ **Do not use ethanol to wash the beads !**
8. Flick the beads to resuspend, then return the tube to the magnetic stand and allow the beads to pellet.
9. Remove the supernatant using a pipette and discard.
10. Spin down and place the tube back on the magnetic stand.
11. Pipette off any residual supernatant.
12. Allow to dry for ~30 seconds, but do not dry the pellet to the point of cracking.
13. Remove the tube from the magnetic stand and resuspend the pellet in **25 µl Elution Buffer (EB).**
14. Spin down and incubate **for 10-20 minutes at 37˚C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution**.
15. Pellet the beads on a magnetic stand until the eluate is clear and colourless
16. Remove and retain **25 µl** of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
17. Quantify 1 µl of eluted sample using a Quantus fluorometer (or Qubit).

Step F. Priming and loading the PromethION Flow Cell

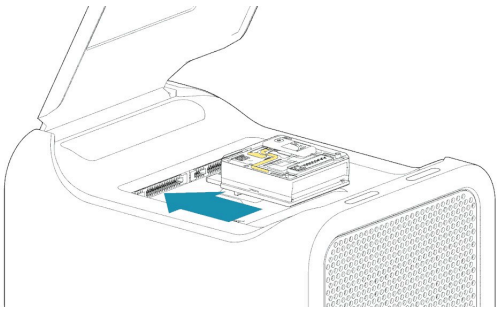
※ **Prewarm :** Ligation Sequencing Kit contents (**SQK-NBD114.96**)
a. Sequencing Buffer (SB)
b. Library Beads (LIB) – or Library Solution (LIS) for viscous libraries
c. Flow Cell Tether (FCT)
d. Flow Cell Flush (FCF)
thaw at RT, mix by vortexing, spin down, store on ice

※ Flow cell: only **R10.4.1 FLO-PRO114M**. Take it out of the fridge **20 minutes** before inserting it (room temperature). Inspect the gold connector pins for condensation (wipe with a lint-free wipe); the black heat pad must be on the underside.

1. Prepare the flow cell priming mix in a clean tube and mix by vortexing at room temperature:

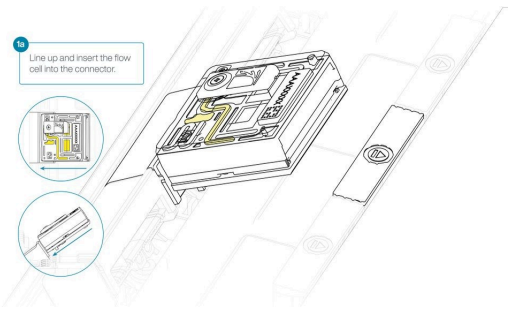
Reagents	**Volume per flow cell**
Flow Cell Flush (FCF)	**1,170 µl**
Flow Cell Tether (FCT)	**30 µl**
Total volume	**1,200 µl**

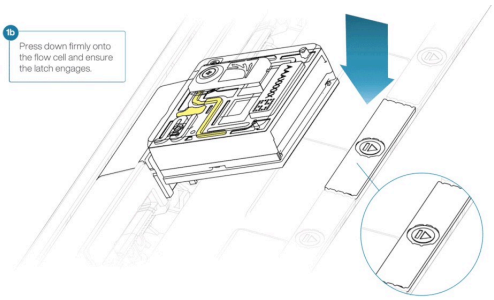
2. Load the flow cell into the device.
PromethION 2 Solo / 2 Integrated: place the flow cell flat on the metal plate and slide it into the docking port until the gold pins or green board cannot be seen.



PromethION 24/48: line up the flow cell with the connector horizontally and vertically, insert smoothly, then press down firmly until the latch clicks into place.

※ **Inserting the flow cell at the wrong angle damages the pins of the PromethION.**

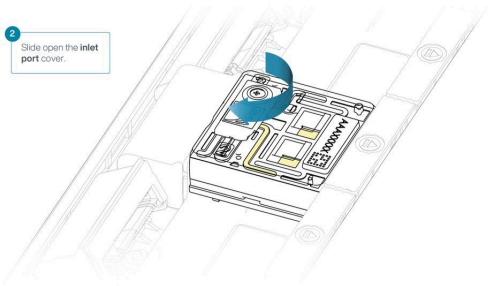




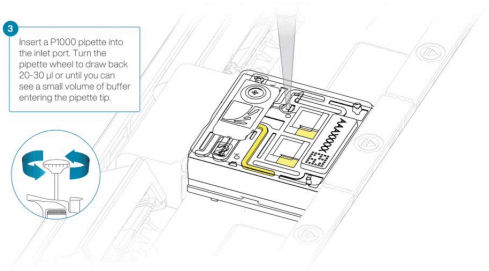
※ Complete a **flow cell check** before loading the library (omit if the flow cell was checked previously).

3. Slide the inlet port cover **clockwise** to open.

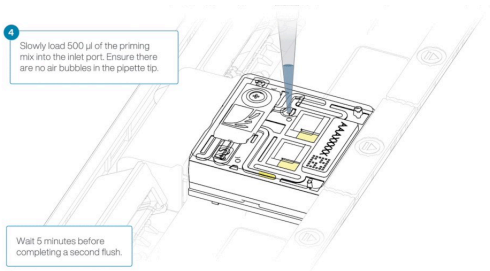
※ **Do not draw back more than 20-30 µl, and keep the array of pores covered by buffer at all times.** Air bubbles in the array irreversibly damage pores.



4. Draw back a small volume to remove any air bubbles:
   1). Set a P1000 pipette to **200 µl**.
   2). Insert the tip into the inlet port.
   3). Turn the wheel until the dial shows **220-230 µl**, or until a small volume of buffer enters the tip.



5. Load **500 µl** of the priming mix into the flow cell via the inlet port, avoiding air bubbles. **Wait five minutes** – meanwhile prepare the library (steps 6-7).

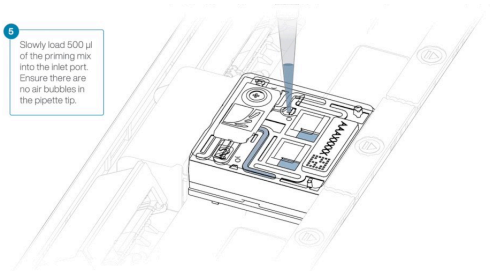


6. Thoroughly mix the Library Beads (LIB) by pipetting **immediately before use** – the beads settle very quickly.

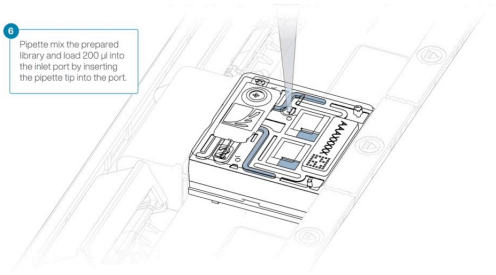
7. In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

Component	**Volume per flow cell**
Sequencing Buffer (SB)	**100 µl**
Library Beads (LIB), mixed before use (or LIS)	**68 µl**
DNA library (all of the eluate from Step E)	**25 µl**
Total volume	**193 µl**

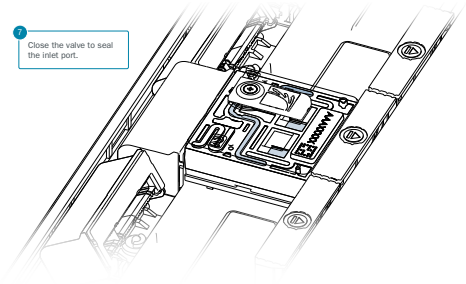
8. Complete the flow cell priming: **slowly** load the second **500 µl** of the priming mix into the inlet port.



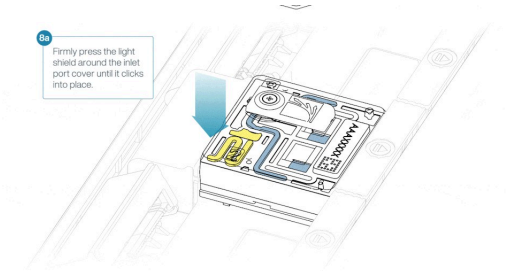
9. Mix the prepared library gently by pipetting up and down just prior to loading, then load **all 193 µl** of library into the inlet port using a P1000 pipette.

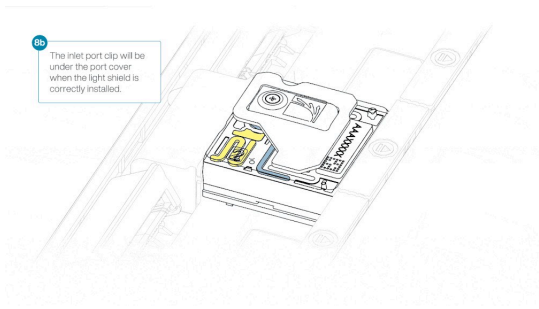


10. Close the valve to seal the inlet port.



11. **Install the light shield as soon as the library has been loaded.** Align the inlet-port cut-out of the light shield with the inlet port cover (leading edge above the flow cell ID), then press firmly around the inlet port cover until the clip clicks into place underneath it.





12. Close the PromethION lid. Wait **at least 10 minutes** after loading before starting the run in MinKNOW (Kit: **SQK-NBD114.96**).

※ After the run: wash the flow cell as soon as possible (Flow Cell Wash Kit EXP-WSH004, lab wash protocol) and store it at 2–8°C.
※ Library storage: DNA LoBind tube, **4°C** short term / re-loading; **-80°C** for > 3 months.