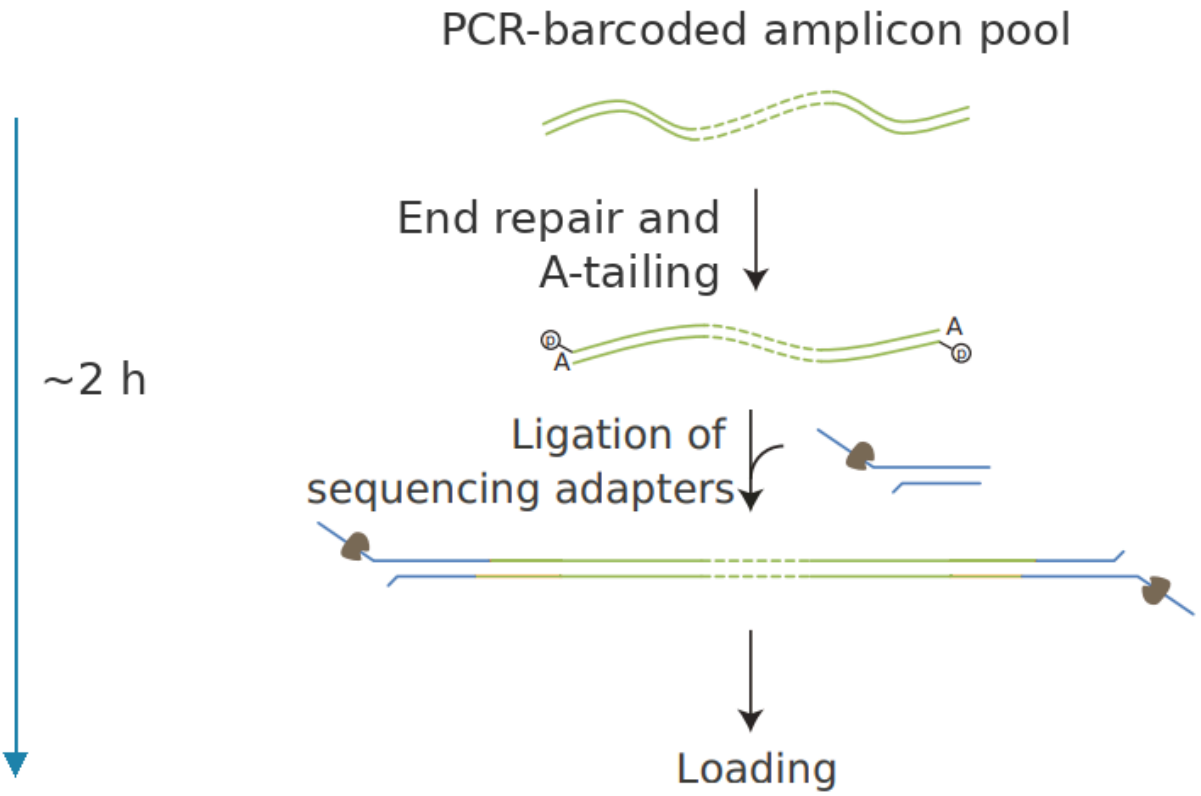
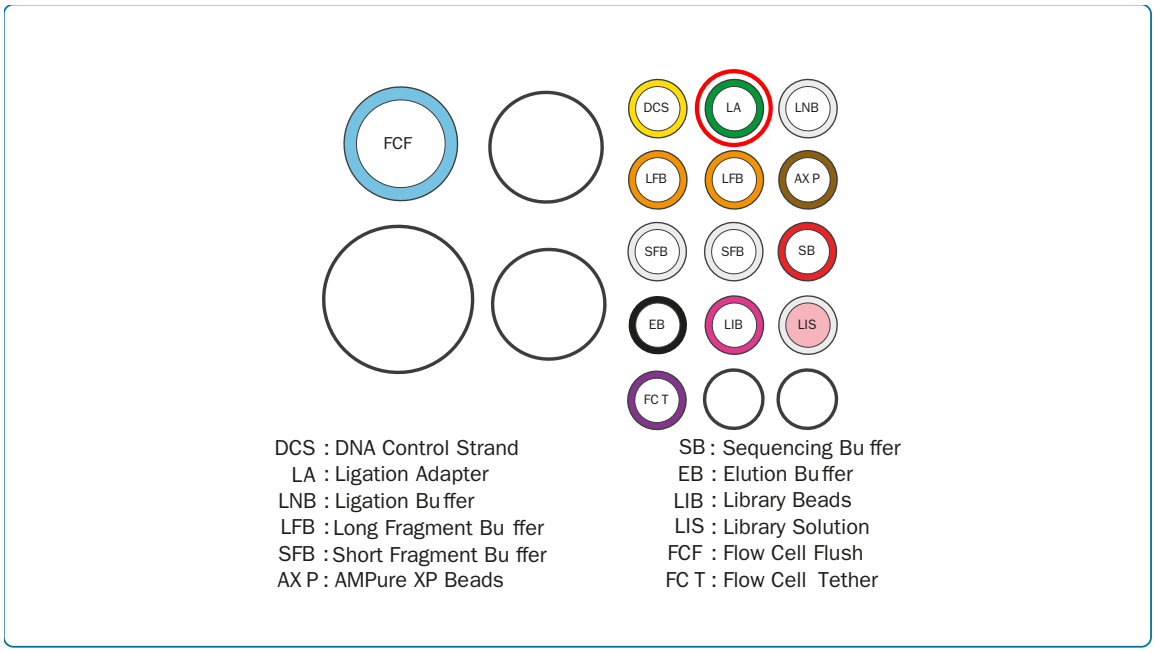
KAPA HyperPrep_SQK-LSK114

(0.5X reagent, PCR-barcoded samples)



SQK-LSK114 kit



DCS : DNA Control Strand
LA : Ligation Adapter
LNB : Ligation Buffer
LFB : Long Fragment Buffer
SFB : Short Fragment Buffer
AXP : AMPure XP Beads
SB : Sequencing Buffer
EB : Elution Buffer
LIB : Library Beads
LIS : Library Solution
FCF : Flow Cell Flush
FCT : Flow Cell Tether

Source: ONT SQK-LSK114 PromethION protocol GDE_9161_v114_revAC_24Sep2025, p. 9; fill volumes: ONT store (2026-09-17).

Fill volume per vial (µl): **LA 40** · LNB 200 · DCS 35 · AXP 1,200 · LFB 1,800 × 2 · SFB 1,800 × 2 SB 700 · EB 500 · LIB 600 · LIS 600 · FCT 200 · FCF 8,000 (one bottle)

From this kit the library prep uses only the **Ligation Adapter (LA)**; clean-up and loading use LFB/SFB, EB, SB, LIB, FCF, FCT. Ligation runs in **KAPA Ligation Buffer + KAPA DNA Ligase**; the ONT Ligation Buffer (LNB) is not used (ONT recommends LNB for LA – test it if yield is low).

KAPA HyperPrep kits

Kapa/*Roche* Kit Codes and Components		
KK8500 *07962312001* **KK8501*** *07962339001* 8 libraries	End Repair & A-Tailing Buffer End Repair & A-Tailing Enzyme Ligation Buffer DNA Ligase KAPA HiFi HotStart ReadyMix (2X)* Library Amplification Primer Mix (10X)*	60 µL 25 µL 245 µL 80 µL 250 µL 50 µL
KK8502 *07962347001* **KK8503*** *07962355001* 24 libraries	End Repair & A-Tailing Buffer End Repair & A-Tailing Enzyme Ligation Buffer DNA Ligase KAPA HiFi HotStart ReadyMix (2X)* Library Amplification Primer Mix (10X)*	210 µL 90 µL 900 µL 300 µL 690 µL 138 µL
KK8504 *07962363001* **KK8505*** *07962371001* 96 libraries	End Repair & A-Tailing Buffer End Repair & A-Tailing Enzyme Ligation Buffer DNA Ligase KAPA HiFi HotStart ReadyMix (2X)* Library Amplification Primer Mix (10X)*	930 µL 400 µL 3.8 mL 1.26 mL 3.0 mL 600 µL

Source: KAPA HyperPrep Kit Technical Data Sheet KR0961 v7.19 (Roche), p. 1.

Compatibility of this protocol

This protocol should only be used in combination with:

1. KAPA HyperPrep kit
2. Ligation Sequencing Kit (**SQK-LSK114**)
3. R10.4.1 PromethION Flow Cells (**FLO-PRO114M**)

Materials :

1. **PCR-barcoded amplicons (pooled)** for R10.4.1 flow cells
2. KAPA HyperPrep kit
3. Ligation Sequencing Kit (**SQK-LSK114**)

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

PCR-barcoded samples: pool all amplicons, then take ~200 fmol of the pool
(ONT input: <1 kb 200 fmol; 1–10 kb 100–200 fmol. 200 fmol = 0.124 ng × bp)
每個樣品樣本量參考表 KAPA HyperPrep 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 HyperPrep kit on ice before use
c. End Repair & A-Tailing Buffer: **thaw at RT and vortex (precipitates)**

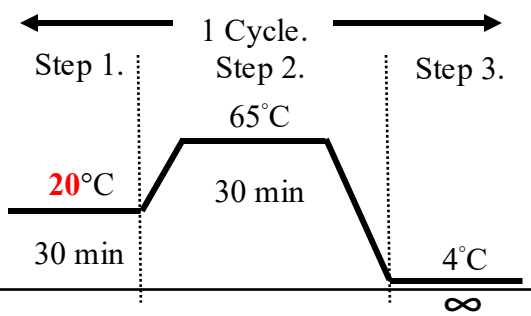
1. Make up the **PCR-barcoded pool (200 fmol)** to **25 µl** using nuclease-free water. Mix gently by pipetting and spin down

3. Combine the following components per tube/well:

Component	KAPA HyperPrep kit
DNA sample **(PCR-barcoded pool)**	**25 µl (200 fmol)**
End Repair & A-Tailing Buffer	**3.5 µl**
End Repair & A-Tailing Enzyme Mix	**1.5 µl**
Total volume	**30 µl**
(200 fmol = 0.124 ng × length in bp;	e.g. 1 kb = 124 ng, 5 kb = 618 ng)

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. Adapter Ligation and clean-up	
※ **Prewarm : SQK-LSK114 kit – Ligation Adapter (LA)** **KAPA HyperPrep kit** – Ligation Buffer (thaw at RT, vortex) & DNA Ligase	
1. In a 1.5 ml Eppendorf LoBind tube, mix in the following order:	
Component (one PCR-barcoded pool)	**Volume**
End-prepped DNA **(from Step A)**	**30 µl** (whole Step A reaction)
Ligation Adapter (LA)	**2.5 µl**
Nuclease-Free water	**2.5 µl**
KAPA Ligation Buffer (not ONT LNB)	**15 µl**
KAPA DNA Ligase	**5 µl**
Total volume	**55 µl**
2. Mix thoroughly by gently pipetting the reaction up and down several times.	
3. Incubate at 20°C for **15 mins**	

Step C. DNA library clean-up

※ **Prewarm : KAPA HyperPure beads**
a. beads at RT; vortex to resuspend
SQK-LSK114 kit at RT before use
b. Long Fragment Buffer (LFB)
c. Short Fragment Buffer (SFB)
d. Elution Buffer (EB)

1. Add **55 µl (1X) of resuspended HyperPure beads** to the **55 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 D. Priming and loading the PromethION Flow Cell

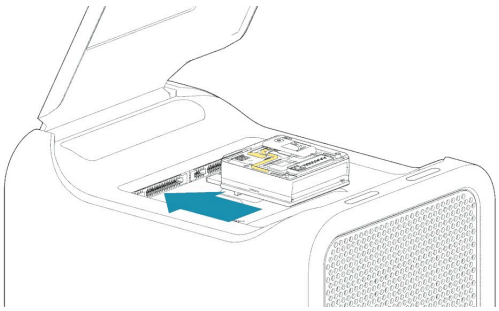
※ **Prewarm :** Ligation Sequencing Kit contents (**SQK-LSK114**)
a. Sequencing Buffer (SB)
b. Library Beads (LIB) – or Library Solution (LIS) for viscous libraries
c. Flow Cell Tether (FCT)
d. Flow Cell Flush (FCF)
thaw at RT, mix by vortexing, spin down, store on ice

※ 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:

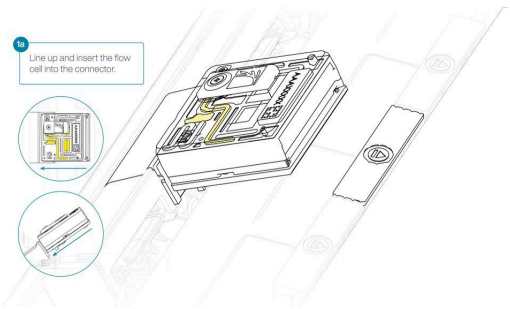
Reagents	**Volume per flow cell**
Flow Cell Flush (FCF)	**1,170 µl**
Flow Cell Tether (FCT)	**30 µl**
Total volume	**1,200 µl**

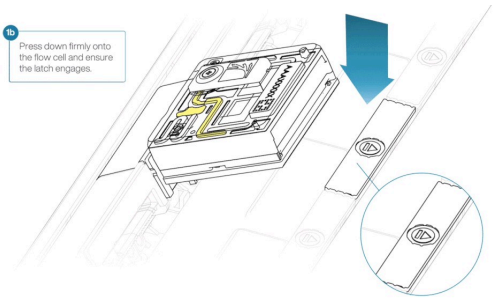
2. Load the flow cell into the device.
PromethION 2 Solo / 2 Integrated: place the flow cell flat on the metal plate and slide it into the docking port until the gold pins or green board cannot be seen.



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.**

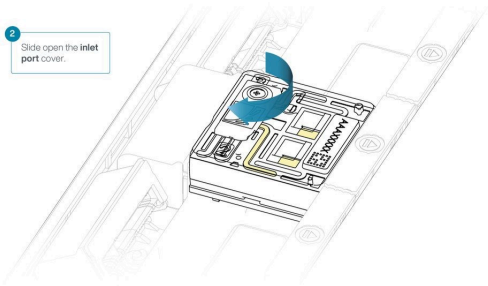




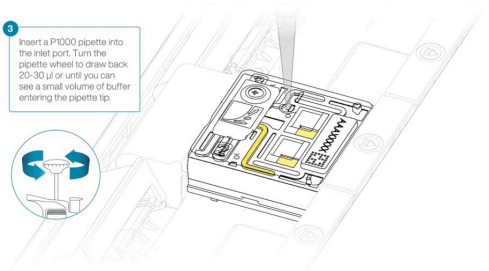
※ Complete a **flow cell check** before loading the library (omit if the flow cell was checked previously).

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

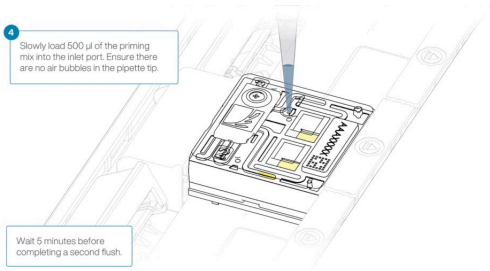
※ **Do not draw back more than 20-30 µl, and keep the array of pores covered by buffer at all times.** Air bubbles in the array irreversibly damage pores.



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).

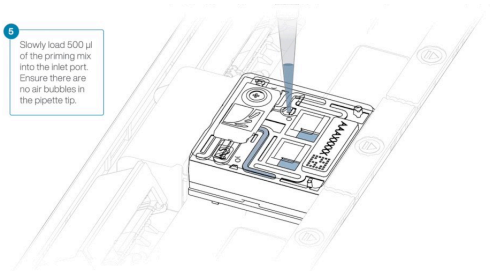


6. Thoroughly mix the Library Beads (LIB) by pipetting **immediately before use** – the beads settle very quickly.

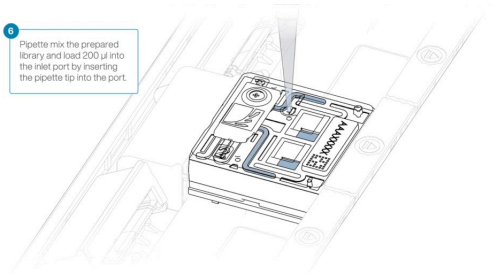
7. In a new 1.5 ml Eppendorf DNA LoBind tube, prepare the library for loading as follows:

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 C)	**25 µl**
Total volume	**193 µl**

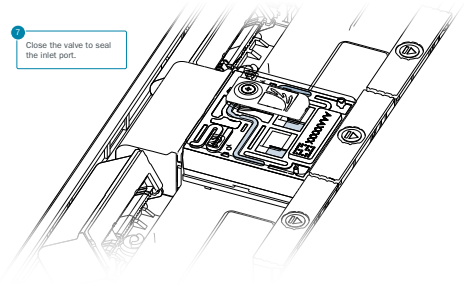
8. Complete the flow cell priming: **slowly** load the second **500 µl** of the priming mix into the inlet port.



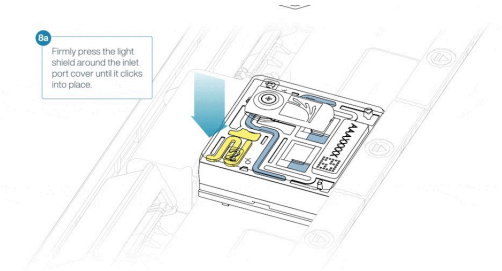
9. Mix the prepared library gently by pipetting up and down just prior to loading, then load **all 193 µl** of library into the inlet port using a P1000 pipette.

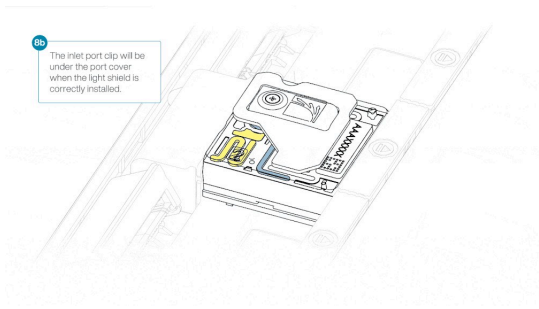


10. Close the valve to seal the inlet port.



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-LSK114**).

※ 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.