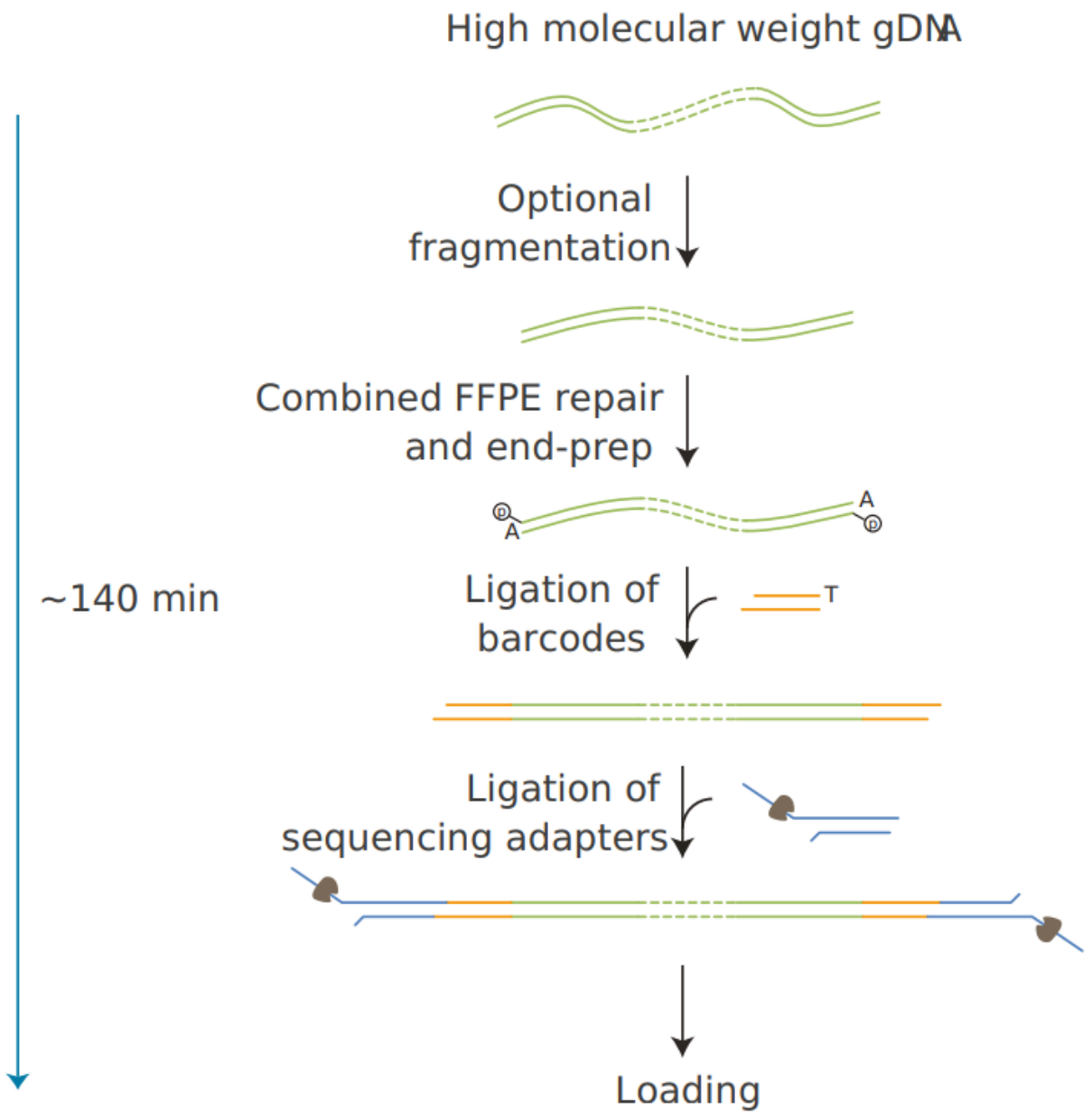
KAPA EvoPrep_SQK-NBD114.24

(0.5X reagent)



SQK-NBD114.24 kit

NA: Native Adapter
SB: Sequencing Buffer
LIB: Library Beads
LIS: Library Solution
EB: Elution Buffer
AXP: AMPure XPBeads
LFB: Long Fragment Buffer
SFB: Short Fragment Buffer
FCF: Flow Cell Flush
FCT: Flow Cell Tether
EDTA: EDTA
DCS: DNAControl Sample
NB01: Native Barcode 1
NB02: Native Barcode 2
NB03: Native Barcode 3
NB04: Native Barcode 4
NB05: Native Barcode 5
NB06: Native Barcode 6
NB07: Native Barcode 7
NB08: Native Barcode 8
NB09: Native Barcode 9
NB10: Native Barcode 10
NB11: Native Barcode 11
NB12: Native Barcode 12
NB13: Native Barcode 13
NB14: Native Barcode 14
NB15: Native Barcode 15
NB16: Native Barcode 16
NB17: Native Barcode 17
NB18: Native Barcode 18
NB19: Native Barcode 19
NB20: Native Barcode 20
NB21: Native Barcode 21
NB22: Native Barcode 22
NB23: Native Barcode 23
NB24: Native Barcode 24

Name	Acronym	Cap colour	No. of vials	Fill volume per vial (µl)
Native Barcodes	NB01-24	Clear	24 (one per barcode)	20
DNA Control Sample ⓘ	DCS	Yellow	2	35
Native Adapter	NA	Green	1	40
Sequencing Buffer	SB	Red	1	700
Library Beads	LIB	Pink	1	600
Library Solution	LIS	White cap, pink label	1	600
Elution Buffer	EB	Black	1	500
AMPure XP Beads	AXP	Amber	4	1,200
Long Fragment Buffer	LFB	Orange	1	1,800
Short Fragment Buffer	SFB	Clear	1	1,800
EDTA	EDTA	Clear	1	700
Flow Cell Flush	FCF	Blue	6	1,170
Flow Cell Tether	FCT	Purple	1	200

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-NBD114.24)
3. FLO-MIN114 (R10.4.1) flow cells

Materials :

1. DNA for every sample to be barcoded for R10.4.1 flow cells
2. KAPA EvoPrep kit
3. Ligation Sequencing Kit (SQK-NBD114.24)

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. SpotON flow cell
6. HyperPure beads (KAPA)

Equipment：

1. Hula mixer (Rotor mixer)
2. MinION Mk1B
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

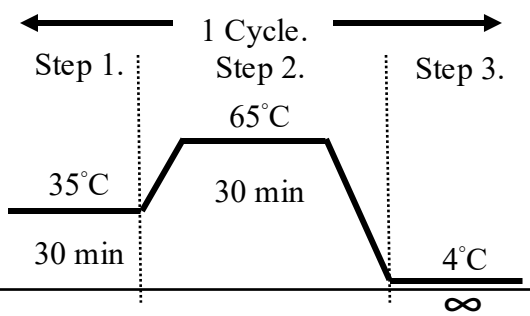
1. Make up each sample to **17.5 µl** using nuclease-free water. Mix gently by pipetting and spin down

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-NBD114.24 kit**
Native barcode

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 (NB01-24)	**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 **30 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 30 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-NBD114.24 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-NBD114.24 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 **15 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 15 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).
Loading 100 fmol of final prepared library onto the flow cells.

Step F. Priming and loading

※ **Prewarm : Ligation Sequencing Kit contents (SQK-NBD114.24)**

a. Sequencing Buffer (SB)
b. Loading Beads (LIB)
c. Flow Cell Tether (FCT)
d. one tube of Flow Cell Flush (FCF)
e. Library Solution (LIS)

1. Vortex each tube of the **Sequencing Buffer (SQB), Flush Tether (FLT)** and **Flush Buffer (FB)** and spin down at RT, respectively.

Step B. Instrument assembly

2. To **prepare the flow cell priming mix** ~~**with BSA**~~, add the following reagents directly to the tube of Flow Cell Flush (FCF), and mix by inverting the tube and pipette mix at room temperature:

Reagents	**Volume**
~~**Bovine Serum Albumin (BSA) at 50 mg/ml**~~	~~5 µl **(final concentration 0.2 mg/ml)**~~
Flow Cell Tether (FCT)	30 µl
One tube of Flow Cell Flush (FCF)	1,170 µl
Total volume	1,200 µl

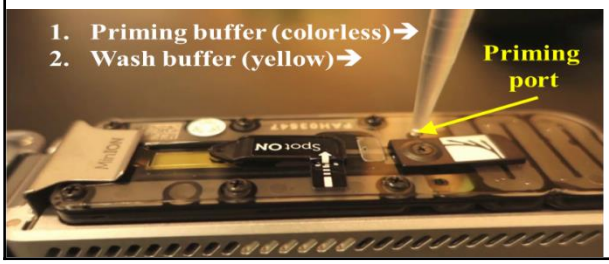
2. Open the MinION Mk1B lid and slide the flow cell under the clip.

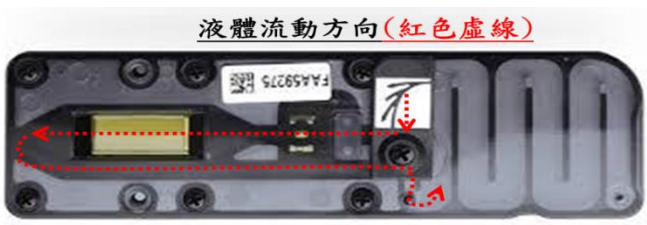
3. Press down firmly on the flow cell to ensure correct thermal and electrical contact.
According to the figures below :

4. Slide the priming port cover clockwise to open the priming port.
According to the figures below :

5. After opening the **priming port**, check for a small air bubble under the cover.
Draw back a small volume to remove any bubbles (a few µl):
1). Set a P1000 pipette to 200 µl
2). Insert the tip into the priming port
3). Turn the wheel until the dial shows 220-230 µl, or until you can see a small volume of buffer entering the pipette tip. Visually check that there is continuous buffer from the priming port across the sensor array.

7. Load 800 µl of the **priming mix** into the flow cell via the priming port, avoiding the introduction of air bubbles. **Wait for 5 minutes**. During this time, prepare the library





For loading by following the steps below :

8. **Thoroughly mix the contents of the Loading Beads (LIB) by pipetting .**
   The Loading Beads (LIB) tube **contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.**

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

Component	**Volume**
DNA library	12 µl (100 fmol)
Sequencing Buffer (SB)	37.5 µl
Loading Beads (LIB), **mixed before use**	25.5 µl
Total volume	75 µl

10. Complete the flow cell priming:
    1). Gently lift the SpotON sample port cover to make the SpotON sample port accessible.
    2). Load 200 µl of the **priming mix** into the flow cell via the **priming port (not the SpotON sample port), avoiding the introduction of air bubbles**.

※Load the library as soon as possible after this step.

11. **Open both priming port and SpotON sample port before loading the sample with loading beads**

11. Mix the prepared library gently by pipetting up and down just prior to loading. Add 75 µl of sample to the flow cell via the **SpotON sample port** in a **dropwise fashion**.

12. Ensure each drop flows 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 priming port and replace the MinION Mk1B lid**.

14. Connect MinION device to the computer, and start basecalling