Nanopore protocol (with EXP-NBD104 and SQK-LSK109)

DNA repair and end-prep (~35mins)

1. Prepare 250 ng DNA into a E-tube
2. In a PCR tube, mix followings :

DNA	24 ul
NEBNext FFPE DNA Repair Buffer	1.75 ul
NEBNext FFPE DNA Repair Mix	1 ul
Ultra II End-prep reaction Buffer	1.75 ul
Ultra II End-prep enzyme Mix	1.5 ul
Total	**30 ul**

3. Mix gently by flicking tube, and spin down
4. Incubate at 20℃ for 5 mins and 65℃ for 5 mins
5. Add 30 ul of resuspended AMPure XP beads to the end-prep reaction and mix by flicking the tube.

   (AMPure XP bead clean-up step : This step can be omitted for simplicity and to reduce library preparation time. However, it has been observed that omission of this step can reduce subsequent adapter ligation efficiency)
6. Incubate on a rotary mixer for 5 mins at RT
7. Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.
8. Keep the tube on the magnet and wash the beads with 200 ul of freshly-prepared 70% EtOH without disturbing the pellet. Remove the EtOh using a pipette and discard.
9. Repeat the previous step
10. Spin down and place the tube back on the magnet. Pipette off any residual EtOH.

    (Allow to dry for ~30sec, but do not dry the pellet to the point of cracking.)
11. Remove the tube from the magnetic rack and resuspend the pellet in 12.5 ul nuclease-free water. Incubate for 2 mins at RT
12. Pellet the beads on a magnet until the eluate is clear and colourless.
13. Remove and retain 12.5 ul of eluate into a tube
14. Quantify 1 ul of eluted sample using a Qubit fluorometer

Native barcode ligation (~110mins)

1. 125 ng of each end-prepped sample to be barcoded to 11.25 ul in nuclease-free water
2. Add the reagents in the order given below (mixing by flicking the tube between each sequential addition) :

125 un end-prepped DNA	11.25 ul
Native Barcode	1.25 ul
Blunt/TA Ligase Master Mix	12.5 ul
Total	**25 ul**

(Mix well by pipetting. Alternatively, mix gently by flicking the tube, and spin down.)

3. Incubate the reaction for 10 mins at RT
4. Add 25 ul of resuspended AMPure XP beads to the end-prep reaction and mix by flicking the tube.
5. Incubate on a rotary mixer for 5 mins at RT
6. Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.
7. Keep the tube on the magnet and wash the beads with 200 ul of freshly-prepared 70% EtOH without disturbing the pellet. Remove the EtOH using a pipette and discard.
8. Repeat the previous step
9. Spin down and place the tube back on the magnet. Pipette off any residual EtOH.

   (Allow to dry for ~30sec, but do not dry the pellet to the point of cracking.)
10. Remove the tube from the magnetic rack and resuspend the pellet in 13 ul nuclease-free water. Incubate for 2 mins at RT
11. Pellet the beads on a magnet until the eluate is clear and colourless.
12. Remove and retain 13 ul of eluate into a tube
13. Quantify 1 ul of eluted sample using a Qubit fluorometer
14. Pool equimolar amounts of each barcoded sample into a tube, ensuring that sufficient sample is combined to produce a pooled sample of 250 ng total.
15. Quantify 1 ul of pooled and barcoded DNA using a Qubit fluorometer
16. Dilute 250 ng pooled sample to 32.5 ul in nuclease-free water.

    (If 250 ng pooled sample exceed 32.5 ul in volume, perform an AMPure clean-up to pooled sample volume, eluting in 32.5 ul of nuclease-free water.)

Adapter ligation and clean-up

1. Taking the pooled and barcoded DNA, perform adapter ligation as follows (Mixing by flicking the tube between each sequential addition) :

250 ng pooled barcoded sample	32.5 ul
Adapter Mix II (AMII)	2.5 ul
NEBNext Quick Ligation Reaction Buffer (5X)	10 ul
Quick T4 DNA ligase	5 ul
Total	**50 ul**

(Mix gently by flicking the tube, and spin down

2. Incubate the reaction for 10 mins at RT
3. Add 25 ul of resuspended AMPure XP beads to the end-prep reaction and mix by flicking the tube.
4. Incubate on a rotary mixer for 5 mins at RT
5. Spin down the sample and pellet on a magnet. Keep the tube on the magnet, and pipette off the supernatant.
6. Wash the beads by adding either 125 ul Long Fragment Buffer (LFB) or 125 ul Short Fragment Buffer (SFB). Flicking beads to resuspend, then return the tube to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard.

   (Long fragments: 3kb or longer)
7. Repeat the previous step
8. Spin down and place the tube back on the magnet. Pipette off any residual supernatant.

   (Allow to dry for ~30sec, but do not dry the pellet to the point of cracking.)
9. Remove the tube from the magnetic rack and resuspend the pellet in 7 ul Elution Buffer (EB). Incubate for 10 mins at RT

   (high molecular weight DNA, incubating at 37˚C can improve the recovery of long franments.
10. Pellet the beads on a magnet until the eluate is clear and colourless.
11. Remove and retain 7 ul of eluate into a tube

    (The prepared library is used for loading into the flow cell. Store the library on ice until ready to load)
12. Quantify 1 ul of eluted sample using a Qubit fluorometer

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Loading the Flongle flow cell

1. In a tube, mix 117 ul of FB with 3ul FLT and mix by pipetting (Priming fluid)
2. To prime your flow cell with the mix of FB and FLT that was prepared earlier, ensure that there is no air gap in the sample port or the pipette tip.
3. Prepare the library in a tube for loading the flongle, as follows :

Sequencing Buffer (SQB)	13.5 ul
Loading Beads (LB)	11 ul
DNA library	5.5 ul
Total	**30 ul**

(LB; mixed immediately before use)

4. To add the Sequencing Mix to the flow cell, ensure that there is no air gap in the sample port or the pipette tip.
5. Seal the Flongle flow cell using the adhesive on the seal tab.

Barcode :

Barcode	큐빅 농도(1)	2	3