MinION

Oxford Nanopore Technologies

Ligation sequencing gDNA - Native Barcoding Kit 24 V14 (SQK-NBD114.24)

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Last update: 7/2/2025

Kit batch number ..... **Flow cell number** ..... **DNA Samples** .....

Checklist: DNA repair and end-prep

Materials

- [ ] 400 ng gDNA per barcode
- [ ] OR 1000 ng gDNA per sample if using ≤4 barcodes
- [ ] AMPure XP Beads (AXP)
- [ ] DNA Control Sample (DCS)

Consumables

- [ ] NEBNext® FFPE DNA Repair Mix (NEB, M6630)
- [ ] NEBNext® Ultra II End Repair/dA-tailing Module (NEB, E7546)
- [ ] Freshly prepared 80% ethanol in nuclease-free water
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Eppendorf™, 0030129504) with heat seals
- [ ] OR 0.2 ml thin-walled PCR tubes
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Qubit dsDNA HS Assay Kit (Invitrogen, Q32851)

Equipment

- [ ] P1000 pipette and tips
- [ ] P200 pipette and tips
- [ ] P100 pipette and tips
- [ ] P20 pipette and tips
- [ ] P10 pipette and tips
- [ ] P2 pipette and tips
- [ ] Multichannel pipette and tips
- [ ] Thermal cycler
- [ ] Microplate centrifuge, e.g. Fisherbrand™ Mini Plate Spinner Centrifuge (Fisher Scientific,11766427)
- [ ] Microfuge
- [ ] Ice bucket with ice
- [ ] Magnetic separation rack
- [ ] Vortex mixer
- [ ] Hula mixer (rotator mixer)
- [ ] Qubit fluorometer (or equivalent)

DNA repair and end-prep	Notes / Observations

For samples containing long gDNA fragments, we recommend using wide-bore pipette tips for the mixing steps to preserve the DNA length.

1. Thaw the AMPure XP Beads (AXP) and DNA Control Sample (DCS) at room temperature and mix by vortexing. Keep the beads at room temperature and store the DNA Control Sample (DCS) on ice.

2. Prepare the NEBNext FFPE DNA Repair Mix and NEBNext Ultra II End Repair / dA-tailing Module reagents in accordance with manufacturer's instructions, and place on ice.

For optimal performance, NEB recommend the following:

1. Thaw all reagents on ice.

2. Flick and/or invert the reagent tubes to ensure they are well mixed.
   Note: Do not vortex the FFPE DNA Repair Mix or Ultra II End Prep Enzyme Mix.

3. Always spin down tubes before opening for the first time each day.

4. The Ultra II End Prep Reaction Buffer and FFPE DNA Repair Buffer may have a little precipitate. Allow the mixture to come to room temperature and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for 30 seconds to solubilise any precipitate.
   Note: It is important the buffers are mixed well by vortexing.

5. The FFPE DNA Repair Buffer may have a yellow tinge and is fine to use if yellow.

Do not vortex the NEBNext FFPE DNA Repair Mix or NEBNext Ultra II End Prep Enzyme Mix.

It is important that the NEBNext FFPE DNA Repair Buffer and NEBNext Ultra II End Prep Reaction Buffer are mixed well by vortexing.

Check for any visible precipitate; vortexing for at least 30 seconds may be required to solubilise any precipitate.

3. Dilute your DNA Control Sample (DCS) by adding 105 µl Elution Buffer (EB) directly to one DCS tube. Mix gently by

pipetting and spin down.

One tube of diluted DNA Control Sample (DCS) is enough for 140 samples. Excess can be stored at -20°C in the freezer.

> **We recommend using the DNA Control Sample (DCS) in your library prep for troubleshooting purposes. However, you can omit this step and make up the extra 1 µl with your sample DNA.**

4. In clean 0.2 ml thin-walled PCR tubes (or a clean 96-well plate), prepare your DNA samples:
   - For >4 barcodes, aliquot 400 ng per sample
   - For ≤4 barcodes, aliquot 1000 ng per sample
5. Make up each sample to 11 µl using nuclease-free water. Mix gently by pipetting and spin down.
6. Combine the following components per tube/well:

Between each addition, pipette mix 10 - 20 times.

Reagent	Volume
DNA sample	11 µl
Diluted DNA Control Sample (DCS)	1 µl
NEBNext FFPE DNA Repair Buffer	0.875 µl
Ultra II End-prep Reaction Buffer	0.875 µl
Ultra II End-prep Enzyme Mix	0.75 µl
NEBNext FFPE DNA Repair Mix	0.5 µl
Total	**15 µl**

> **We recommend making up a mastermix of the End Prep and DNA Repair reagents for the total number of samples and adding 3 µl to each well.**

7. Ensure the components are thoroughly mixed by pipetting and spin down in a centrifuge.
8. Using a thermal cycler, incubate at 20°C for 5 minutes and 65°C for 5 minutes.
9. Transfer each sample into a clean 1.5 ml Eppendorf DNA LoBind tube.
10. Resuspend the AMPure XP beads (AXP) by vortexing.
11. Add 15 µl of resuspended AMPure XP Beads (AXP) to each end-prep reaction and mix by flicking the tube.

12. Incubate on a Hula mixer (rotator mixer) for 5 minutes at room temperature.

13. Prepare sufficient fresh 80% ethanol in nuclease-free water for all of your samples. Allow enough for 400 µl per sample, with some excess.

14. Spin down the samples and pellet the beads on a magnet until the eluate is clear and colourless. Keep the tubes on the magnet and pipette off the supernatant.

15. Keep the tube on the magnet and wash the beads with 200 µl of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard.

If the pellet was disturbed, wait for beads to pellet again before removing the ethanol.

16. Repeat the previous step.

17. Briefly spin down and place the tubes back on the magnet for the beads to pellet. Pipette off any residual ethanol. Allow to dry for 30 seconds, but do not dry the pellets to the point of cracking.

18. Remove the tubes from the magnetic rack and resuspend the pellet in 10 µl nuclease-free water. Spin down and incubate for 2 minutes at room temperature.

19. Pellet the beads on a magnet until the eluate is clear and colourless.

20. Remove and retain 10 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.
    - Dispose of the pelleted beads

Quantify 1 µl of each eluted sample using a Qubit fluorometer.

Take forward an equimolar mass of each sample to be barcoded forward into the native barcode ligation step. However, you may store the samples at 4°C overnight.

Checklist: Native barcode ligation

Materials

- [ ] Native Barcodes (NB01-24)
- [ ] AMPure XP Beads (AXP)
- [ ] EDTA (EDTA)
- [ ] Short Fragment Buffer (SFB)

Consumables

- [ ] NEB Blunt/TA Ligase Master Mix (NEB, M0367)
- [ ] Nuclease-free water (e.g. ThermoFisher, AM9937)
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] Eppendorf twin.tec® PCR plate 96 LoBind, semi-skirted (Eppendorf™, 0030129504) with heat seals
- [ ] OR 0.2 ml thin-walled PCR tubes
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Qubit™ dsDNA HS Assay Kit (ThermoFisher, Q32851)

Equipment

- [ ] Magnetic separation rack
- [ ] Vortex mixer
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Microfuge
- [ ] Thermal cycler
- [ ] Ice bucket with ice
- [ ] Multichannel pipette and tips
- [ ] P1000 pipette and tips
- [ ] P200 pipette and tips
- [ ] P100 pipette and tips
- [ ] P20 pipette and tips
- [ ] P10 pipette and tips
- [ ] P2 pipette and tips
- [ ] Qubit™ fluorometer (or equivalent for QC check)

Native barcode ligation

1. **Prepare the NEB Blunt/TA Ligase Master Mix according to the manufacturer's instructions, and place on ice:**
   1. Thaw the reagents at room temperature.
   2. Spin down the reagent tubes for 5 seconds.
   3. Ensure the reagents are fully mixed by performing 10 full volume pipette mixes.
2. **Thaw the EDTA at room temperature and mix by vortexing. Then spin down and place on ice.**
3. **Thaw the Short Fragment Buffer (SFB) at room temperature and mix by vortexing. Place on ice.**

Notes / Observations

4. Thaw the Native Barcodes (NB01-24) at room temperature. Briefly spin down, individually mix the barcodes required for your number of samples by pipetting, and place them on ice.

5. Select a unique barcode for each sample to be run together on the same flow cell. Up to 24 samples can be barcoded and combined in one experiment.

Please note: Only use one barcode per sample.

6. In clean 0.2 ml PCR-tubes or a 96-well plate, add the reagents in the following order per well:

Between each addition, pipette mix 10 - 20 times.

Reagent	Volume
End-prepped DNA	7.5 µl
Native Barcode (NB01-24)	2.5 µl
Blunt/TA Ligase Master Mix	10 µl
Total	**20 µl**

7. Thoroughly mix the reaction by gently pipetting and briefly spinning down.

8. Incubate for 20 minutes at room temperature.

9. Add the following volume of EDTA to each well and mix thoroughly by pipetting and spin down briefly.

Note: Ensure you follow the instructions for the cap colour of your EDTA tube.

EDTA cap colour	Volume per well
For clear cap EDTA	2 µl
For blue cap EDTA	4 µl

EDTA is added at this step to stop the reaction.

10. Pool all the barcoded samples in a 1.5 ml Eppendorf DNA LoBind tube.

Note: Ensure you follow the instructions for the cap colour of your EDTA tube.

	Volume per sample	For 6 samples	For 12 samples	For 24 samples
Total volume for preps using **clear cap EDTA**	22 µl	132 µl	264 µl	528 µl

	Volume per sample	For 6 samples	For 12 samples	For 24 samples
Total volume for preps using **blue cap EDTA**	24 µl	144 µl	288 µl	576 µl

We recommend checking the base of your tubes/plate are all the same volume before pooling and after to ensure all the liquid has been taken forward.

11 Resuspend the AMPure XP Beads (AXP) by vortexing.

12 Add 0.4X AMPure XP Beads (AXP) to the pooled reaction, and mix by pipetting.

Note: Ensure you follow the instructions for the cap colour of your EDTA tube.

	Volume per sample	For 6 samples	For 12 samples	For 24 samples
Volume of AXP for preps using **clear cap EDTA**	9 µl	53 µl	106 µl	211 µl
Volume of AXP for preps using **blue cap EDTA**	10 µl	58 µl	115 µl	230 µl

13 Incubate on a Hula mixer (rotator mixer) for 10 minutes at room temperature.

For optimal results we recommend using Short Fragment Buffer (SFB) for the clean-up steps following native barcoding.

Our development teams have determined that using the **Short Fragment Buffer (SFB) instead of ethanol** for the post-barcoding washes removes excess barcode more efficiently. This translates to improved barcode classification rates and reduced physical barcode cross-talk.

New batches of the native barcoding kits will contain sufficient Short Fragment Buffer (SFB) to follow the updated method. If you have an older format of the native barcoding kit with a lower volume of Short Fragment Buffer (SFB), you may require additional reagents available through the SFB Expansion (EXP-SFB001).

Please note, the old method using 80% ethanol is still compatible with this method. If you wish to continue using 80% ethanol for your post-barcoding wash, please follow the steps below:

- Prepare sufficient fresh 80% ethanol in nuclease-free water for your washes.
- Use the freshly prepared 80% ethanol in place of the Short Fragment Buffer (SFB) for the wash steps below.

14 Spin down the sample and pellet on a magnet for 5 minutes. Keep the tube on the magnetic rack until the eluate is clear and colourless, and pipette off the supernatant.

15 Keep the tube on the magnetic rack and wash the beads with 700 µl of Short Fragment Buffer (SFB) without disturbing the pellet. Remove the buffer using a pipette and discard.

If the pellet was disturbed, wait for beads to pellet again before removing the buffer.

16 Repeat the previous step.

17 Spin down and place the tube back on the magnetic rack. Pipette off any residual buffer.

18 Remove the tube from the magnetic rack and resuspend the pellet in 35 µl nuclease-free water by gently flicking.

19 Incubate for 10 minutes at 37°C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution.

20 Pellet the beads on a magnetic rack until the eluate is clear and colourless.

21 Remove and retain 35 µl of eluate into a clean 1.5 ml Eppendorf DNA LoBind tube.

Quantify 1 µl of eluted sample using a Qubit fluorometer.

Take forward the barcoded DNA library to the adapter ligation and clean-up step. However, you may store the sample at 4°C overnight.

Checklist: Adapter ligation and clean-up

Materials

- [ ] Long Fragment Buffer (LFB)
- [ ] Short Fragment Buffer (SFB)
- [ ] Elution Buffer (EB)
- [ ] Native Adapter (NA)
- [ ] AMPure XP Beads (AXP)

Consumables

- [ ] NEBNext® Quick Ligation Module (NEB, E6056)
- [ ] 1.5 ml Eppendorf DNA LoBind tubes
- [ ] Qubit™ Assay Tubes (Invitrogen, Q32856)
- [ ] Qubit™ dsDNA HS Assay Kit (ThermoFisher, Q32851)

Equipment

- [ ] Microfuge
- [ ] Magnetic separation rack
- [ ] Vortex mixer
- [ ] Hula mixer (gentle rotator mixer)
- [ ] Thermal cycler
- [ ] P1000 pipette and tips
- [ ] P200 pipette and tips
- [ ] P100 pipette and tips
- [ ] P20 pipette and tips
- [ ] P10 pipette and tips
- [ ] Ice bucket with ice
- [ ] Qubit™ fluorometer (or equivalent for QC check)

Adapter ligation and clean-up

The Native Adapter (NA) used in this kit and protocol is not interchangeable with other sequencing adapters.

Check your flow cell.

We recommend performing a flow cell check before starting adapter ligation and clean-up to ensure you have a flow cell with sufficient pores for a good sequencing run.

See the flow cell check instructions in the MinKNOW protocol for more information.

1 **Prepare the NEBNext Quick Ligation Reaction Module according to the manufacturer's instructions, and place on ice:**

1. Thaw the reagents at room temperature.
2. Spin down the reagent tubes for 5 seconds.
3. Ensure the reagents are fully mixed by performing 10 full volume pipette mixes. **Note:** Do NOT vortex the Quick T4

Notes / Observations

DNA Ligase.

The NEBNext Quick Ligation Reaction Buffer (5x) may have a little precipitate. Allow the mixture to come to room temperature and pipette the buffer up and down several times to break up the precipitate, followed by vortexing the tube for several seconds to ensure the reagent is thoroughly mixed.

Do not vortex the Quick T4 DNA Ligase.

2. Spin down the Native Adapter (NA) and Quick T4 DNA Ligase, pipette mix and place on ice.

3. Thaw the Elution Buffer (EB) at room temperature and mix by vortexing. Then spin down and place on ice.

Depending on the wash buffer (LFB or SFB) used, the clean-up step after adapter ligation is designed to either enrich for DNA fragments of >3 kb, or purify all fragments equally.

- To enrich for DNA fragments of 3 kb or longer, use Long Fragment Buffer (LFB)
- To retain DNA fragments of all sizes, use Short Fragment Buffer (SFB)

4. Thaw either Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) at room temperature and mix by vortexing. Then spin down and keep at room temperature.

5. In a 1.5 ml Eppendorf LoBind tube, mix in the following order:

Between each addition, pipette mix 10 - 20 times.

Reagent	Volume
Pooled barcoded sample	30 µl
Native Adapter (NA)	5 µl
NEBNext Quick Ligation Reaction Buffer (5X)	10 µl
Quick T4 DNA Ligase	5 µl
Total	**50 µl**

6. Thoroughly mix the reaction by gently pipetting and briefly spinning down.

7. Incubate the reaction for 20 minutes at room temperature.

The next clean-up step uses Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB) rather than 80% ethanol to wash the beads. The use of ethanol will be detrimental to the sequencing reaction.

8. Resuspend the AMPure XP Beads (AXP) by vortexing.
9. Add 20 µl of resuspended AMPure XP Beads (AXP) to the reaction and mix by pipetting.
10. Incubate on a Hula mixer (rotator mixer) for 10 minutes at room temperature.
11. Spin down the sample and pellet on the magnetic rack. Keep the tube on the magnet and pipette off the supernatant.
12. Wash the beads by adding either 125 µl Long Fragment Buffer (LFB) or Short Fragment Buffer (SFB). Flick the beads to resuspend, spin down, then return the tube to the magnetic rack and allow the beads to pellet. Remove the supernatant using a pipette and discard.
13. Repeat the previous step.
14. Spin down and place the tube back on the magnet. Pipette off any residual supernatant.
15. Remove the tube from the magnetic rack and resuspend pellet in 15 µl Elution Buffer (EB).
16. Spin down and incubate for 10 minutes at 37°C. Every 2 minutes, agitate the sample by gently flicking for 10 seconds to encourage DNA elution.
17. Pellet the beads on a magnet until the eluate is clear and colourless, for at least 1 minute.
18. Remove and retain 15 µl of eluate containing the DNA library into a clean 1.5 ml Eppendorf DNA LoBind tube.

Dispose of the pelleted beads

> **Quantify 1 µl of eluted sample using a Qubit fluorometer.**

19. Depending on your DNA library fragment size, prepare your final library in 12 µl of Elution Buffer (EB).

Fragment library length	Flow cell loading amount
Very short (<1 kb)	100 fmol
Short (1-10 kb)	35–50 fmol
Long (>10 kb)	300 ng

Note: If the library yields are below the input recommendations, load the entire library.

If required, we recommend using a mass to mol calculator such as the NEB calculator.

The prepared library is used for loading onto the flow cell. Store the library on ice or at 4°C until ready to load.

If quantities allow, the library may be diluted in Elution Buffer (EB) for splitting across multiple flow cells.

Depending on how many flow cells the library will be split across, more Elution Buffer (EB) than what is supplied in the kit will be required.

Checklist: Priming and loading the MinION and GridION Flow Cell

Materials	Consumables	Equipment
☐ Flow Cell Flush (FCF)	☐ 1.5 ml Eppendorf DNA LoBind tubes	☐ MinION or GridION device
☐ Flow Cell Tether (FCT)	☐ MinION/GridION Flow Cell	☐ MinION/GridION Flow Cell Light Shield
☐ Library Solution (LIS)	☐ Nuclease-free water (e.g. ThermoFisher, AM9937)	☐ P1000 pipette and tips
☐ Library Beads (LIB)	☐ Bovine Serum Albumin (BSA) (50 mg/ml) (e.g Invitrogen™ UltraPure™ BSA 50 mg/ml, AM2616)	☐ P100 pipette and tips
☐ Sequencing Buffer (SB)		☐ P20 pipette and tips
		☐ P10 pipette and tips

Priming and loading the MinION and GridION Flow Cell	Notes / Observations

Please note, this kit is only compatible with R10.4.1 flow cells (FLO-MIN114).

Priming and loading a flow cell

We recommend all new users watch the 'Priming and loading your flow cell' video before your first run.

1. Thaw the Sequencing Buffer (SB), Library Beads (LIB) or Library Solution (LIS, if using), Flow Cell Tether (FCT) and Flow Cell Flush (FCF) at room temperature before mixing by vortexing. Then spin down and store on ice.

For optimal sequencing performance and improved output on MinION R10.4.1 flow cells (FLO-MIN114), we recommend adding Bovine Serum Albumin (BSA) to the flow cell priming mix at a final concentration of 0.2 mg/ml.

Note: We do not recommend using any other albumin type (e.g. recombinant human serum albumin).

2. To prepare the flow cell priming mix with BSA, combine Flow Cell Flush (FCF) and Flow Cell Tether (FCT), as directed below. Mix by pipetting at room temperature.

Note: We are in the process of reformatting our kits with single-use tubes into a bottle format. Please follow the instructions for your kit format.

Single-use tubes format: Add 5 µl Bovine Serum Albumin (BSA) at 50 mg/ml and 30 µl Flow Cell Tether (FCT) directly to a tube of

Flow Cell Flush (FCF).

Bottle format: In a suitable tube for the number of flow cells, combine the following reagents:

Reagent	Volume per flow cell
Flow Cell Flush (FCF)	1,170 µl
Bovine Serum Albumin (BSA) at 50 mg/ml	5 µl
Flow Cell Tether (FCT)	30 µl
Total volume	**1,205 µl**

3. Open the MinION or GridION device lid and slide the flow cell under the clip. Press down firmly on the priming port cover to ensure correct thermal and electrical contact.

> **Complete a flow cell check to assess the number of pores available before loading the library.**
>
> This step can be omitted if the flow cell has been checked previously.
>
> See the flow cell check instructions in the MinKNOW protocol for more information.

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

> **Take care when drawing back buffer from the flow cell. Do not remove more than 20-30 µl, 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. After opening the priming port, check for a small air bubble under the cover. Draw back a small volume to remove any bubbles:

    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, to draw back 20-30 µl, or until you can see a small volume of buffer entering the pipette tip

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

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

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

7. Thoroughly mix the contents of the Library Beads (LIB) by pipetting.

The Library Beads (LIB) tube contains a suspension of beads. These beads settle very quickly. It is vital that they are mixed immediately before use.

We recommend using the Library Beads (LIB) for most sequencing experiments. However, the Library Solution (LIS) is available for more viscous libraries.

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

Reagent	Volume per flow cell
Sequencing Buffer (SB)	37.5 µl
Library Beads (LIB) mixed immediately before use, or Library Solution (LIS), if using	25.5 µl
DNA library	12 µl
Total	**75 µl**

9. 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 priming port (**not** the SpotON sample port), avoiding the introduction of air bubbles.

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

11. Add 75 µl 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.

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

Install the light shield on your flow cell as soon as library has been loaded for optimal sequencing output.

We recommend leaving the light shield on the flow cell when library is loaded, including during any washing and reloading steps. The shield can be removed when the library has been removed from the flow cell.

13 Place the light shield onto the flow cell, as follows:

1. Carefully place the leading edge of the light shield against the clip. **Note:** Do not force the light shield underneath the clip.

2. Gently lower the light shield onto the flow cell. The light shield should sit around the SpotON cover, covering the entire top section of the flow cell.

The MinION Flow Cell Light Shield is not secured to the flow cell and careful handling is required after installation.

Close the device lid and set up a sequencing run on MinKNOW.

When a flow cell is inserted into the MinION Mk1D, the device lid will sit on top of the flow cell, leaving a small gap around the sides. This is normal and has no impact on the performance of the device.

Please refer to this FAQ regarding the device lid.

Checklist: Flow cell reuse and returns

Materials	Consumables	Equipment
☐ Flow Cell Wash Kit (EXP-WSH004)		

Flow cell reuse and returns	Notes / Observations

1. After your sequencing experiment is complete, if you would like to reuse the flow cell, please follow the Flow Cell Wash Kit protocol and store the washed flow cell at +2°C to +8°C.

The Flow Cell Wash Kit protocol is available on the Nanopore Community.

We recommend you to wash the flow cell as soon as possible after you stop the run. However, if this is not possible, leave the flow cell on the device and wash it the next day.

2. Alternatively, follow the returns procedure to send the flow cell back to Oxford Nanopore.

Instructions for returning flow cells can be found here.

If you encounter issues or have questions about your sequencing experiment, please refer to the Troubleshooting Guide that can be found in this protocol.