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nCoV-2019 McGill Nanopore LibPrep Protocol, 5 ng NB

Forked from nCoV-2019 McGill Nanopore LibPrep Protocol, 10 ng NB

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

Artic nCoV-2019 McGill modified Lunascript Reverse Transcriptase nanopore sequencing protocol.

Native barcoding

1

Barcode the amplicon pools using native barcodes.

> **Note**
>
> This is a ‘one-pot ligation’ protocol for native barcoded ligation libraries. We have seen no reduction in performance compared to standard libraries, and is made faster by using the Ultra II® ligation module which is compatible with the Ultra II® end repair/dA-tailing module removing a clean-up step.

2

Set up the following reaction for each sample:

Component	Volume
DNA amplicons	5 µL
Nuclease-free water	7.5 µL
Ultra II End Prep Reaction Buffer	1.75 µL
Ultra II End Prep Enzyme Mix	0.75 µL
Total	15 µL

3

Incubate at room temperature for 00:10:00

Incubate at 65 °C for 00:05:00

Incubate on ice for 00:01:00

4

Add the following directly to the previous reactions:

Component	Volume
NBXX barcode	2.5 µL
Ultra II Ligation Master Mix	10 µL
Ligation Enhancer	0.3 µL
Water	4.2 µL
Total	17 µL 20 ul total with 3 ul from step 3

5

Incubate at room temperature for 00:15:00

Incubate at 70 °C for 00:10:00

Incubate on ice for 00:01:00

Note

The 70°C incubation is to inactivate the DNA ligase to prevent barcode cross-ligation when reactions are pooled in the next step.

6

Clean-up the native barcodes using the following protocol (**before pooling**):

Add 0.8X of SPRI beads (28.6 ul) to the sample tube and mix gently by pipetting.
Incubate for 5 min at room temperature.
Pellet on magnet for 5 min. Remove supernatant.
Add 200 ul of 80% ethanol to the pellet and wash twice.
Elute in **20 ul** elution buffer.

7

Quantify the barcodes using a fluorimetric dsDNA assay.

8

Normalize the barcodes to **10 ng** each and pool (except neg. ctrl - which will be equal volume).

9

Clean-up the barcode pool using the following protocol.

Add 0.8X of SPRI beads to the sample tube and mix gently by pipetting.
Incubate for 5 min at room temperature.
Pellet on magnet for 5 min. Remove supernatant.
Add 200 ul of 80% ethanol to the pellet and wash twice.
Elute in **30 ul** elution buffer.

10

Set up the following AMII adapter ligation reaction:

Component	Volume
Barcoded amplicon pools	30 µL
NEBNext Quick Ligation Reaction Buffer (5X)	10 µL
AMII adapter mix	5 µL

Quick T4 DNA Ligase	5 µL
Total	50 µL

11

Incubate at room temperature for 00:15:00

12

Clean-up the native barcodes using the following protocol:

Add an equal volume (1:1) of SPRI beads to the sample tube and mix gently by pipetting.
Incubate for 5 min at room temperature.
Pellet on magnet for 5 min. Remove supernatant.
Add 200 ul of **SFB** to the pellet and **resuspend beads completely by pipette mixing**.
Pellet on beads, remove supernanant, and repead the wash step with another 200 ul of **SFB**.
Elute in 15 ul **EB (provided in the ONT kit)**.
Incubate at room temperature for 00:02:00
Place on magnetic rack.
Transfer final library to a new 1.5 mL Eppendorf tube.

Note

SFB will remove excess adapter without damaging the adapter-protein complexes. Do not use 70% ethanol as in early clean-ups.

13 Quantify the final library using a fluorimetric dsDNA assay. (e.g. : Pico Green with a 0-100ng standard Curve)

Note

Final library can be now be stored in 10 mM Tris pH 8 at 4°C for up to a week if needed otherwise proceed directly to MinION sequencing.

14 Prime the flowcell and load 20 ng to 40 ng sequencing library onto the flowcell. Dilute library in EB if required.

Note

The original protocol says 20 ng, but this leads to only ~50% pore occupancy. Loading 40 ng leads to ~70% pore occupancy but the flow cell needs to be refueled.

14.1

Thaw the following reagents at room temperature before placing on ice:

Sequencing buffer (SQB)
Loading beads (LB)
Flush buffer (FLB)
Flush tether (FLT)

14.2

Add 30 µL FLT to the FLB tube (1.16 mL) and mix well by vortexing.

14.3

If required place a new MinION flowcell onto the MinION by flipping open the lip and pushing one end of the flowcell under the clip and pushing down gently.

14.4

Rotate the inlet port cover clockwise by 90° so that the priming port is visible.

14.5

Take a P1000 pipette and tip and set the volume to 800 µL . Place the tip in the inlet port and holding perpendicularly to the plane of the flowell remove any air from the inlet port by turning the volume dial anti-clockwise.

Note

Be careful not to remove so much volume that air is introduced onto the rectangular array via the outlet.

14.6

Load 800 µL of FLB (plus FLT) into the flow cell via the inlet port, dispense slowly and smoothly trying to avoid the introduction of any air bubbles.

14.7

Wait for 00:05:00

14.8

Gently lift the SpotON cover to open the SpotON port.

14.9

Load another 200 µL of FLB (plus FLT) into the flow cell via the inlet port, this will initiate a siphon at the SpotON port to allow you to load the library dilution.

14.10

In a new tube prepare the library dilution for sequencing:

Component	Volume
SQB	37.5 µL
LB	25.5 µL
Final library	12 µL
Total	75 µL

14.11

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

14.12

Add the 75 µL library dilution to the flow cell via the SpotON sample port in a dropwise fashion. Ensure each drop siphons into the port before adding the next.

14.13

Gently replace the SpotON sample port cover, making sure the bung enters the SpotON port, close the inlet port and close the MinION lid.

15

Start the sequencing run using MinKNOW.

15.1

If required plug the MinION into the computer and wait for the MinION and flowcell to be detected.

15.2

Choose flow cell 'FLO-MIN106' from the drop-down menu.

15.3

Then select the flowcell so a tick appears.

15.4

Click the 'New Experiment' button in the bottom left of the screen.

15.5

On the New experiment popup screen, select the running parameters for your experiment from the individual tabs:

Experiment: Name the run in the experiment field, leave the sample field blank.

Kit: Selection: Select LSK109 as there is no option for native barcoding (NBD104).

Run Options: Set the run length to 6 hours (you can stop the run once sufficient data has been collected as determined using RAMPART).

Basecalling: Leave basecalling turned but select 'fast basecalling'.

Output: The number of files that MinKNOW will write to a single folder. By default this is set to 4000 but can be reduced to make RAMPART update more frequently.

Click 'Start run'.

15.6

Monitor the progress of the run using the MinKNOW interface.