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One-pot native barcoding of amplicons v3 (LoCost)

Josh Quick[1]

[1]University of Birmingham

1 Works for me This protocol is published without a DOI.

Josh Quick

ABSTRACT

This one-pot native barcoding protocol was developed in conjunction with Oxford Nanopore Technologies, New England Biolabs and BCCDC.

EXTERNAL LINK

http://lab.loman.net/protocols/

ATTACHMENTS

One-pot native barcoding protocol (1).pdf

PROTOCOL CITATION

Josh Quick 2020. One-pot native barcoding of amplicons v3 (LoCost). **protocols.io** https://protocols.io/view/one-pot-native-barcoding-of-amplicons-v3-locost-bh44j8yw

EXTERNAL LINK

http://lab.loman.net/protocols/

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PARENT PROTOCOLS

In steps of

nCoV-2019 sequencing protocol v3 (LoCost)

SAFETY WARNINGS

See SDS (Safety Data Sheet) for safety warnings and hazards.

1 In a new PCR strip-tube/plate set up the following reaction for each sample:

Component	Volume
PCR dilution from previous step	3.3 µL
Ultra II End Prep Reaction Buffer	1.2 µL
Ultra II End Prep Enzyme Mix	0.5 µL
Nuclease-free water	5 µL

Total	**10 µL**

Make a master mix of end-preparation reagents and nuclease-free water and aliquot into strip-tube/plate to improve reproducability.

2 Incubate at room temperature for **00:15:00**

Incubate at **65 °C** for **00:15:00**

Incubate on ice for **00:01:00**

3 In a new PCR strip-tube/plate set up the following reaction for each sample:

Component	Volume
End-preparation reaction mixture	0.75 µL
NBXX barcode	1.25 µL
Blunt/TA Ligase Master Mix	5 µL
Nuclease-free water	3 µL
Total	**10 µL**

Use one native barcode from the EXP-NBD104 (1-12), EXP-NBD114 (13-24) or EXP-NBD196 per sample. Use 12 or more barcodes per library or there will be insufficient total material to achieve good yields.

4 Incubate at room temperature for **00:20:00**

Incubate at **65 °C** for **00:10:00**

Incubate on ice for **00:01:00**

The 65°C incubation is to inactivate the DNA ligase to prevent barcode cross-ligation when reactions are pooled in the next step.

5 In a new **1.5 mL** Eppendorf tube pool all one-pot barcoding reactions together.

If processing 12-24 samples pool all **10 µl** from each native barcoding reaction.

if processing 48 samples pool **5 µl** from each native barcoding reaction.

If processing 96 samples pool **2.5 µl** from each native barcoding reaction so as not to exceed a pool volume of **240 µl** which would make the clean-up volume too large.

6 Add 0.4x volume of SPRI beads to the sample tube and mix gently by either flicking or pipetting. For example add **96 µl** SPRI beads to **240 µl** pooled one-pot barcoding reactions.

0.4x volume of SPRI is sufficient to bind 400 bp amplicons in the presence of ligation buffer, do not use 1x as this will result in an excessive large bead pellet.

7 Mix by vortexing and pulse centrifuge to collect all liquid at the bottom of the tube. Incubate for **00:05:00** at room temperature.

8 Place on magnetic rack and incubate for **00:02:00** or until the beads have pelleted and the supernatant is completely clear. Carefully remove and discard the supernatant, being careful not to touch the bead pellet.

9 Add **250 µl** SFB and resuspend beads completely by pipette mixing. Pulse centrifuge to collect all liquid at the bottom of the tube and place on the magnet. Remove supernatant and discard.

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

10 Repeat steps 11.9 to perform a second SFB wash. Pulse centrifuge and remove any residual SFB.

You do not need to allow to air dry with SFB washes.

11 Add **200 µl** of room-temperature [M]**70 % volume** ethanol to bathe the pellet. Carefully remove and discard ethanol, being careful not to touch the bead pellet.

Only perform 1x 70% ethanol wash

12 Pulse centrifuge to collect all liquid at the bottom of the tube and carefully remove as much residual ethanol as possible using a P10 pipette.

13 With the tube lid open incubate for **00:01:00** or until the pellet loses it's shine (if the pellet dries completely it will crack and become difficult to resuspend).

14 Resuspend pellet in **30 µl** **10 Milimolar (mM)** Tris pH 8.0, mix gently by either flicking or pipetting and incubate for **00:02:00** .

15 Place on magnet and transfer sample to a clean **1.5 mL** Eppendorf tube ensuring no beads are transferred into this tube.