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Version 2

One-pot native barcoding of amplicons v4 (LoCost) V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

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

Attachments



One-pot native barco...

64KB

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

Note

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

- 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
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Note

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.

If processing <11 samples, increase quantities in the above reaction to allow for sufficient material for sequencing. For example: if processing 6 samples, double the component volumes for a final reaction volume of 20μL for each sample.

4 Incubate at room temperature for 00:20:00

Incubate at 65 °C for 00:10:00

Incubate on ice for 00:01:00

Note

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.

Note

If processing <24 samples pool the total volume from all barcodes.

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.

Note

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.

Note

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.

Note

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

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

**Note**

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 its shine (if the pellet dries completely it will crack and become difficult to resuspend).
- 14 Resuspend pellet in  30 μL  10 millimolar (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.