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One-pot native barcoding of amplicons v2

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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

PDF

One-pot native barco...

64KB

Safety warnings

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

1 Set up the following reaction for each sample:

Component	Volume
PCR dilution from previous step	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

2 Incubate at room temperature for 00:10:00
Incubate at 65 °C for 00:10:00
Incubate on ice for 00:01:00

3 In a new 1.5mL Eppendorf tube set up the following reaction:

Component	Volume
Previous reaction mixture	1.5 µL
Nuclease-free water	5.7 µL
NBXX barcode	2.5 µL
Ultra II Ligation Master Mix	10 µL
Ligation Enhancer	0.3 µL
Total	20 µL

Note

Use one native barcode from the EXP-NBD104 (1-12) or EXP-NBD114 (13-24) per sample. Use from 6 to 24 barcodes in a library, any fewer and 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

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 20 µL one-pot barcoding reactions together.

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 12-plex pooled one-pot native barcoding reactions.

Note

0.4x volume of SPRI will only bind 400 bp amplicons in the presence of ligation buffer as in a one-pot reaction, do not use 1x as this will result in excessive native barcode carryover.

7 Pulse centrifuge to collect all liquid at the bottom of the tube.

8 Incubate for 00:05:00 at room temperature.

9 Place on magnetic rack and incubate for 00:02:00 or until the beads have pelleted and the supernatant is completely clear.

10 Carefully remove and discard the supernatant, being careful not to touch the bead pellet.

11 Add 700 µl SFB and resuspend beads completely by pipette mixing.

Note

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

12 Pulse centrifuge to collect all liquid at the bottom of the tube.

13 Remove supernatant and discard.

14 Repeat steps 11-13 to perform a second SFB wash.

15 Pulse centrifuge and remove any residual SFB.

Note

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

16 Add 200 µl of room-temperature 70 % volume ethanol to bathe the pellet.

17 Carefully remove and discard ethanol, being careful not to touch the bead pellet.

Note

Only perform 1x 70% ethanol wash

18 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.

19 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).

20 Resuspend pellet in 30 µL Elution Buffer (EB), mix gently by either flicking or pipetting and incubate for 00:02:00 .

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