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One-pot native barcoding of amplicons (Ultra II AMII ligation) [↗](#)

Forked from [One-pot native barcoding of amplicons](#)

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1 Works for me [dx.doi.org/10.17504/protocols.io.bdaq2dw](https://doi.org/10.17504/protocols.io.bdaq2dw)

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ABSTRACT

This is a 'one-pot ligation' protocol for Oxford Nanopore native barcoded ligation libraries using shearing.



This protocol has been slightly modified from Josh Quick's original to use the Ultra II ligation for barcoding *and* adaptor ligation

EXTERNAL LINK

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

ATTACHMENTS

[One-pot native barcoding protocol \(1\).pdf](#)

GUIDELINES

Scope:

There has been no evidence of a reduction of performance compared to standard libraries, yet it can be 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.

The FFPE DNA repair step is optional. If you have the time, we recommend using the double incubation times in **bold**. If you are in a hurry, the times in *italic* are a good compromise between speed and efficiency.

Required:

g-TUBEs (optional)
SQK-LSK108 1D ligation kit
Native barcoding kit
Ultra II End Repair/dA-Tailing Module
Ultra II Ligation Module
FFPE DNA Repair Mix (optional)
Ampure XP beads
80% ethanol
EB (10 mM Tris-HCl pH 8)

SAFETY WARNINGS

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

- 1 Set up the following reaction for each sample:

Component	Volume
DNA amplicons (5ng)	12.5 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 00:05:00

Incubate on ice for 00:01:00

- 3 Add the following directly to the previous reactions:

Component	Volume
NBXX barcode	2.5 µl
Ultra II Ligation Master Mix	17.5 µl
Ligation Enhancer	0.5 µl
Total	35.5 µl



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 00:15:00

Incubate at 70 °C for 00:10:00

Incubate on ice for 00:01:00



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






- 5 Pool all barcoded fragments together into a new 1.5 ml Eppendorf tube and perform a SPRI bead clean-up. Elute in 45ul.



PAUSE POINT: As long as you have not yet ligated the sequencing adapter, the library can be stored at 4 °C and continue with the prep at a later point. It is better to store at 4°C, as freezing and thawing can introduce nicks or breaks in the DNA. Several days to weeks in the fridge are possible. For longer-term storage, the library can be placed at -20 °C, though unnecessary freeze-thaw cycles should be avoided for best results.

6 Quantify the barcoded amplicon pools using the Quantus Fluorometer using the ONE dsDNA assay.

7 Set up the following AMII adapter ligation reaction:

Component	Volume
Barcoded amplicon pools	 44 µl
AMII adaptor	 5 µl
Ultra II Ligation Master Mix	 50 µl
Ligation Enhancer	 1 µl
Total	 100 µl



The input of barcoded amplicon pools will depend on the number of barcoded pools and should be between 40 ng (8 barcodes) and 120 ng (24 barcodes).

8 Incubate at room temperature for  00:20:00

9 Add  100 µl (1:1) of SPRI beads to the sample tube and mix gently by either flicking or pipetting.



Vortex SPRI beads thoroughly before use to ensure they are well resuspended, the solution should be a homogenous brown colour.

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

11 Incubate at  37 °C for  00:10:00 .

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

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

14 Add  200 µl SFB and resuspend beads completely by pipette mixing.






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

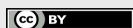
15 Pulse centrifuge to collect all liquid at the bottom of the tube.

- 16 Remove supernatant and discard.
- 17 Repeat steps 14-16 to perform a second SFB wash.
- 18 Pulse centrifuge and remove any residual SFB.



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

- 19 Add  **13 µl** EB and resuspend beads by pipette mixing.
- 20 Incubate at  **37 °C** for  **00:10:00**.
- 21 Place on magnetic rack.
- 22 Transfer final library to a new 1.5mL Eppendorf tube.



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