Mar 07, 2020

One-pot native barcoding of amplicons (Ultra II AMII ligation)

Forked from One-pot native barcoding of amplicons

Josh Quick[1], Kirstyn Brunker[2]

[1]University of Birmingham, [2]University of Glasgow

1 Works for me dx.doi.org/10.17504/protocols.io.bdaqi2dw

Kirstyn Brunker

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.