Adapter ligation with AMII v2

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

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ABSTRACT

This is a subprotocol for performing adapter ligation with AMII

EXTERNAL LINK

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

ATTACHMENTS

One-pot native barcoding protocol (1).pdf

PROTOCOL CITATION

Josh Quick 2020. Adapter ligation with AMII v2. **protocols.io** https://protocols.io/view/adapter-ligation-with-amii-v2-bkaqksdw

EXTERNAL LINK

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

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41008

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 **1.5 µl** Eppendorf tube set up the following AMII adapter ligation reaction.

Component	Volume
Barcoded amplicon pool	30 µL
NEBNext Quick Ligation Reaction Buffer (5X)	10 µL
Adapter Mix (AMII)	5 µL
Quick T4 DNA Ligase	5 µL

Total	50 µL

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

3 Add **50 µl** (1:1) of SPRI beads to the sample tube and mix gently by either flicking or pipetting. Pulse centrifuge to collect all liquid at the bottom of the tube.

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

There will be some variation in clean-up efficiencies but expect to carry around 50% through this clean-up

4 Incubate for **00:05:00** at room temperature.

5 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.

6 Add **250 µl** SFB and resuspend beads completely by pipette mixing. Pulse centrifuge to collect all liquid at the bottom of the tube. 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.

7 Repeat steps 13.6 to perform a second SFB wash.

8 Pulse centrifuge and remove any residual SFB. Add **15 µl** EB (ONT) and resuspend beads by pipette mixing.

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

9 Incubate at room temperature for **00:02:00** .

10 Place on magnetic rack until clear. Transfer final library to a new 1.5mL Eppendorf tube.