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Adapter ligation with AMX

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1 Works for me dx.doi.org/10.17504/protocols.io.bdp5i5q6

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ABSTRACT

This is a subprotocol for generating a library from a single amplicon pool

EXTERNAL LINK

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

ATTACHMENTS

One-pot native barcoding protocol (1).pdf

SAFETY WARNINGS

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

1 Set up the following AMX adapter ligation reaction:

Component	Volume
End-repaired amplicon pools	30 µl
Ligation Buffer (LNB)	10 µl
Adapter Mix (AMX)	5 µl
Quick T4 DNA Ligase	5 µl
Total	50 µl

There will be some variation in clean-up efficiencies but expect to carry around 80% through a clean-up.

2 Incubate at room temperature for 00:10:00

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3 Add **50 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.

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

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

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

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

8 Add **250 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.

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

10 Remove supernatant and discard.

11 Repeat steps 14-16 to perform a second SFB wash.

12 Pulse centrifuge and remove any residual SFB.

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

13 Add **15 µl** EB and resuspend beads by pipette mixing.

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

15 Place on magnetic rack.

16 Transfer final library to a new 1.5mL Eppendorf tube.

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