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## Adapter ligation with AMX

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

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


## Attachments



One-pot native barco...






64KB

## 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 $\mu\text{L}$
Ligation Buffer (LNB)	 10 $\mu\text{L}$
Adapter Mix (AMX)	 5 $\mu\text{L}$
Quick T4 DNA Ligase	 5 $\mu\text{L}$
<b>Total</b>	 50 $\mu\text{L}$

#### Note



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


- 3 Add  50  $\mu\text{L}$  (1:1) of SPRI beads to the sample tube and mix gently by either flicking or pipetting.

#### Note

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  $\mu\text{L}$  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.

- 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.

#### Note

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

- 13 Add  15  $\mu\text{L}$  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.