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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

PDF

One-pot native barco...

64KB

Troubleshooting

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

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 µL (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 µL 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 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.