GeneLab Standard Operating Procedure:

Manual Illumina TruSeq total RNA (Ribo Gold) library clean-up from adapter dimers

February 2021

Version 1.0

Document Revisions

Document Number	Revision Number	Date	Description of Changes
XXX	XXX	Month Year	XXX

Scope and Purpose

Protocol written for 48 samples

This SOP describes the steps used by NASA GeneLab for manually cleaning Illumina TruSeq Total RNA libraries of adapter dimers.

Reagents

1. RSB (Resuspension buffer) from Illumina TruSeq total RNA (Ribo Gold)
2. AMPure XP reagent (Beckman Coulter cat#A63882/A638880/A63881)
3. Multi-channel pipettes
4. Rod magnetic stand
5. Liquid reservoirs

Procedure

- *Make sure the AMPure beads and RSB buffer were equilibrated to room temperature for 30min prior beginning the procedure.*

1. Bring all wells to a final volume of 50uL by addition of appropriate volume of RSB.
2. Using a multi-channel pipette and a reservoir, add 50uL of AMPure beads into all library wells. (1X SPRI clean up). Seal the plate.
3. Vortex using the heat-block or a plate vortexer at 1800 RPM for 2 minutes.
4. Incubate at room temperature for 15 minutes.
5. Quick spin the plate and carefully open the plate seal.
6. Place plate on a magnetic rod stand and incubate for 5 minutes or until the liquid is clear.

7. Remove the supernatant.
8. Without removing plate from magnetic stand, wash **two** times as follows:
    a. Using a multi-channel pipette, add 200uL of fresh (<24HR old) EtOH to each well.
    b. Incubate for 30 seconds.
    c. Remove and discard all supernatant from each well.
9. Use a 20uL multi-channel pipette to remove residual EtOH from each well.
10. Air-dry on the magnetic stand for 7-10min. Check for “coffee bean appearance”, avoid over drying.
11. Remove plate from the magnetic stand.
12. Using a multi-channel pipette and a reservoir, add 30uL of RSB in to each well, seal the plate.
13. Vortex using the heat-block or a plate vortexer at 1800 RPM for 2 minutes.
14. Incubate at room temperature for 2 minutes.
15. Quick spin the plate and carefully open the plate seal.
16. Place the plate on a magnetic stand for 5 minutes.
17. Transfer 30uL of supernatant into clean wells.