# 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 |
|---|---|---|---|
| GL-SOP-6.6 | 1.0 | Feb 27 2019 | Original Document |
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# 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.