

<b>GeneLab SOP for manual Illumina TruSeq total RNA (Ribo Gold) library clean-up from adapter dimers</b>	Document No.:	GL-SOP-6.6
	Version:	2.0
	Created:	02 27 2019
	Last revised:	02 17 2021
	Last revised by:	Valery Boyko

### **Purpose/Scope:**

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 in to 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.