GeneLab SOP for manual Illumina TruSeq total RNA (Ribo Gold) library clean-up from adapter dimers	Document No.:	GL-SOP-6.6
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	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. 3x 96-well half skirted plates
3. AMPure XP reagent (Beckman Coulter cat#A63882/A638880/A63881)
4. Multi-channel pipettes
5. Rod magnetic stand
6. Liquid reservoirs

Procedure:

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

1. Thaw the original library plate(20uL) and the QC sister plate(10uL).
2. Using a multi-channel pipette, combine the volume of the 2 plates.
3. Choose 5 wells and measure combined volume to make sure it is 30uL. If volume differs, bring all wells to a final volume of 50uL by addition of appropriate volume of RSB. If volume is 30uL, proceed to step 4.
4. Using a multi-channel pipette, add 20uL of RSB.
5. Using a multi-channel pipette and a reservoir, add 40uL of AMPure beads in to all library wells. (0.8X SPRI clean up). Seal the plate.
6. Vortex using the heat-block or a plate vortexer at 1800 RPM for 2 minutes.
7. Incubate at room temperature for 20 minutes (Precise time).
8. Quick spin the plate and carefully open the plate seal.

9. Place plate on a rod magnetic stand and incubate for 5 minutes.
10. 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.
11. Use a 20uL multi-channel pipette to remove residual EtOH from each well.
12. Air-dry on the magnetic stand for 7-10min. Check for “coffee bean appearance”, avoid over drying.
13. Remove plate from the magnetic stand.
14. Using a multi-channel pipette and a reservoir, add 50uL of RSB in to each well, seal the plate.
15. Vortex using the heat-block or a plate vortexer at 1800 RPM for 2 minutes.
16. Incubate at room temperature for 2 minutes.
17. Quick spin the plate and carefully open the plate seal.
18. Place the plate on a magnetic stand for 5 minutes.
19. Transfer 50uL of supernatant into clean wells. (same plate can be used).
20. Repeat steps 5-13 one more time.
21. Using a multi-channel pipette and a reservoir, add 30uL of RSB in to each well, seal the plate.
22. Vortex using the heat-block or a plate vortexer at 1800 RPM for 2 minutes.
23. Incubate at room temperature for 5 minutes.
24. Quick spin the plate and carefully open the plate seal.
25. Place the plate on a magnetic stand for 5-10 minutes.
26. Transfer 30uL of supernatant in a new plate.
27. Make a sister QC plate by transferring 10uL of the libraries in to a new plate.