TruSeq RNA with Ribo-Zero depletion

1. Pipet **112.5 µl Ribo-Zero Magnetic Beads** into a 1.5 ml tube. When pipetting beads do so slowly and carefully.
2. Place in magnetic rack for 1 min.
3. Remove and discard supernatant.
4. Remove tube from rack, add 1 ml H_2O and resuspend beads by pipetting.
5. Repeat steps 2 and 3 one more time.
6. Add **32.5 µl Ribo-Zero Magnetic Bead Resuspension Solution** to each tube.
7. Resuspend beads by pipetting up and down.
8. Optional: Add **0.5 µl RiboGuard RNase Inhibitor** to each tube.
9. Store beads at room temperature.

10. In 0.2 ml tube mix:
    2 µl Ribo-Zero Reaction Buffer
    1 µg total RNA
    5 µl Ribo-Zero rRNA Removal Solution
    H_2O to **20 µl** final volume
11. Incubate at 68˚C for 10 min.
12. Incubate at room temperature for 5 min.
13. Add RNA from step 12 to the beads from step 9. Mix immediately and thoroughly by pipeting to prevent clumping of beads.
14. Incubate at room temperature for 5 min.
15. Mix by vortexing at medium speed for 5 seconds.
16. Incubate at 50˚C for 5 minutes.
17. Immediately place tubes in magnetic rack for 1 min.
18. Carefully remove **42.5 µl** of supernatant and transfer to 1.5 ml tube.

19. Add **42.5 µl AMPure XP beads** and **42.5 µl 30 % PEG_{8000} 1.25 M NaCl**
20. Incubate at room temperature for 15 min.
21. Place on magnetic rack for 5 min.
22. Remove and discard supernatant. When removing supernatant do so very slowly with pipetman being careful not to take any beads.
23. Keep sample in magnetic rack and add 200 µl of freshly prepared 80% ethanol.
24. Incubate for 30 seconds. Remove and discard all supernatant.
25. Repeat steps 7 and 8 one more time.
26. Add **6.5 µl Elute, Prime, Fragment Mix** and pipet up and down until beads are in a homogenous suspension.
27. Incubate at room temperature for 4 min.
28. Place in magnetic rack for 5 min.
29. Transfer **5.67 µl** of the supernatant to a new 0.2 ml PCR tube.
30. Incubate in thermocycler:
    94˚ C – 8 min
    4˚ C – hold
31. Continue at step 27 of TruSeq RNA protocol using 1/3 reagent volumes.