Title	RNA clean up - phenol-chloroform extraction & ETOH precipitation		
Protocol Number	MOLBIO20003	Written by	Duha A.
Adapted from	MO Lab, VU, RG, & Utexas	Version	1.1
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Note: if you're using phenol, you need to make sure it is acidic phenol (it is needed for RNA extraction)
Precool the centrifuge to 4°C

Reagents:

- Acid Phenol:Chloroform
- 100% Alcohol
- 75% Alcohol
- 3M Na Acetate pH 5.5 or 5.2
- Glycoblue

Protocol:

1. Using the final volumes obtained from DNAase treatments, add RNAase free water to achieve total volume of 100µL for each sample.
2. Add the 100µL acid phenol:chloroform to 100µL of RNA.
3. Vortex for 15 seconds
4. Centrifuge at 13,000 RPM for 5 mints at 4°C
5. Collect the supernatant (aqueous phase) in new Eppendorfs

To precipitate the RNA:

6. Add 1/10 volume 3M Na acetate for 100uL sample (= 10µL)
7. Add 2.5 volumes 100% cold ETOH for 100uL sample (= 250µL)
8. Add 2µL of glycoblue
9. Incubate at -20°C for at least 1 hour
10. Spin at 13,000 RPM at 4°C centrifuge for 10-15 minutes
11. Using a pipette, remove the 100% ethanol
12. Add 500µL of cold 75% ethanol to wash the pellet
13. Shake the eppendorfs gently
14. Centrifuge at 13,000 RPM at 4°C centrifuge for 5 minutes
15. Remove the 75% ethanol by decanting or pipetting
16. Repeat the washing step (total of 2 washes)
17. Allow the pellet to airdry for 10-20 mints in 37 °C heat blocks
18. Resuspend the pellet in 22µL of RNAase free water (2µL to account for nanodrop)
19. Dose RNA using the nanodrop and record RNA quantity, 260/280 and 260/230 ratios
20. Store the RNA at -20°C or proceed to cDNA synthesis