Hot Phenol RNA Preparation

General Notes

Always practice RNase-Free technique when working with RNA
- Use RNA-only pipet tips and tubes
- Wipe off counter tops and pipetmen with RNaseZap
- Filter-sterilize solutions prior to use
- Use either DEPC-H$_2$O or MQ-H$_2$O
- Keep RNA cold (4 °C or lower)

**Lysis solution (8x)** [make ~ 10 mL at a time)]
- 320 mM NaAcetate (pH 5.0)
- 8% SDS
- 16 mM EDTA

**Protocol:**

1. **Pellet** bacteria cells by spinning at 4 °C, 5,000 x g, for 5 minutes.
   - a. If growing bacteria in M9 media, pellet *at least* 2 mLs.
   - b. If growing bacteria in LB media, you can skip this step and just take 500 µL of culture directly to Step 4.

2. **Remove** supernatant, being careful not to lose pellet

3. **Resuspend** pellet in 500 µL 1x M9

4. **Add** 71 µL 8x Lysis Buffer, quickly pipetting up and down to mix. *Immediately add* 570 µL Acid Phenol/Chloroform (Ambion), vortex.

5. **Incubate** 5 minutes at 65 °C with regular vortexing (every 50 sec)

6. **Spin** > 12,000 x g, 10 minutes

7. **Extract** with phenol/chloroform at least twice until interface is clean
   - Each time you want to keep the aqueous (top) layer for the next step
   - *Note:* the aqueous layer will continually decrease in volume. Add an equal volume of phenol/chloroform each time. E.g. For 500 µL aqueous layer, add 500 µL phenol/chloroform.

8. After the final extraction, **add** 5 µL glycogen (Ambion) and **precipitate** the RNA in the aqueous layer with 3 volumes of 100% ethanol (-70 °C, 30 minutes)
   - You can also put the tubes on well-crushed ice in the -20 °C freezer.

9. **Spin** 12,000 x g at 4 °C for 10 minutes

10. **Rinse** pellet with 500 µL of 70% ethanol, spin 2 minutes
11. **Air dry** pellet, with tube inverted on Kimwipe, for 10 minutes

12. **Resuspend** pellet in 50 µL DEPC-H₂O

13. **Quantitate** RNA concentrations
   
   Make a 1:10 dilution in H₂O and measure absorbance at 260 and 280 nm (OD₂₆₀ of 1 = 40 µg/mL; 260/280 ratio should be between 1.8 and 2.0)