Hot Phenol RNA Preparation

General Notes

Ref: Massé, E., Escorcia, F.E., Gottesman, S. (2003) *Genes. Dev*. **17**, 2374-2383.

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_2O or MQ-H_2O
- 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 <u>keep the aqueous (top) layer</u> 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-H_2O

13. **Quantitate** RNA concentrations

    Make a 1:10 dilution in H_2O and measure absorbance at 260 and 280 nm (OD_{260} of 1 = 40 µg/mL; 260/280 ratio should be between 1.8 and 2.0)