Phase Lock Gel 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_2O or MQ-H_2O
- Keep RNA cold (4 °C or lower)

Lysis solution (8x) [make ~ 10 mL at a time] – filter sterilize each time before use
320 mM NaAcetate (pH 5.0)
8% SDS
16 mM EDTA

Protocol:

1. **Pellet** bacteria cells by spinning at 4 °C, 5,000 -6,000 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 – 16,000 x g, 10 minutes
7. **Transfer** the top aqueous layer into a pre-spun (12,000 – 16,000 x g for 1-2 min) 2 mL Phase Lock Gel tube. Add an equal volume of (Acid Phenol)/Chloroform and invert gently to mix (do not vortex).
8. **Spin** at 12,000-16,000 x g for 5 minutes to separate phases
9. **Pour** supernatant to a 1.5 mL tube. **Add** 1/10 volume 3 M NaAcetate (pH 5.2-5.6), 5 µL glycogen, and an equal volume of room temperature isopropanol. **Mix** by inversion.
10. **Spin** 12,000 x g at 4 °C for 20 minutes
11. **Rinse** pellet with 200 µL of **ice-cold** 70% ethanol, spin 2-3 minutes. **Repeat**.
12. **Air dry** pellet, with tube inverted on Kimwipe, for 10 minutes
13. **Resuspend** pellet in 100 µL DEPC-H_2O. For more concentrated RNA, decrease to 30-50 µL
14. **Quantify** RNA concentrations

    OD_{260} of 1 = 40 µg/mL; 260/280 ratio should be between 1.8 and 2.0