**RNA Isolation**

1. Grow O/N cultures.
2. Spin down 1 mL of saturated O/N culture.
3. Resuspend cells in 200 uL of Lysis Buffer.
4. Add ~0.2 g glass beads.
5. Add 200 uL Phenol:Chloroform.
6. Vortex for 10 min.
7. Spin 5 min at top speed.
8. Transfer 150 uL from top layer and add to 300 uL 100% ethanol (*RNA quality*). Precipitate RNA in -20°C freezer for at least 30 min.
9. Pellet RNA by spinning for 10 min at top speed. Aspirate ethanol.
10. Add 1 mL of 70% ethanol (*RNA quality*), vortex, and spin for 2 min at top speed. Aspirate ethanol.
11. Resuspend pellet in 50 uL of water (*RNA quality*).
12. Store RNA at -20°C.

**cDNA Synthesis**

1. Add 0.5 uL RNA to 2.5 uL water (*RNA quality*) and 1.0 uL of Random Primer. Vortex. Quick spin to collect liquid at bottom. Incubate for 5 min at 65°C.
   1. Optional: create Master Mix of water and primer (include 10% extra)
   2. Alternative: substitute sequence-specific primer (0.5 uL of 100 uM stock)
2. Let mix cool for 1 min. Add 5.0 uL of Reaction Mix and 1.0 uL of Enzyme Mix. Vortex. Quick spin to collect liquid at bottom. Incubate for 1 hr at 42°C.
   1. Optional: create Master Mix of Reaction Mix and Enzyme Mix (include 10% extra)
3. After 1 hr at 42°C, incubate for 5 min at 80°C. Let mix cool for 5 min. Quick spin to collect liquid at bottom.
4. Store cDNA at -20°C.

**PCR**

1. Add 0.5 uL of cDNA to 2.5 uL Q5 Buffer, 0.1 uL dNTPs (25 mM), 0.625 uL Forward Primer (10 uM), 0.625 uL Reverse Primer (10 uM), 0.125 uL Q5 Enzyme, and 8.1 uL water (molecular grade). Vortex. Quick spin to collect liquid at bottom.
   1. Optional: create Master Mix
   2. To screen for the presence of M1 virus, primers M1 F1 and M1 R6 are recommended.
   3. For downstream sequencing of K1 ORF, primers M1 F5 and M1 R6 are recommended.
   4. To screen for the presence of LA virus, primers LA F2 and LA R2 are recommended.
2. Perform thermal cycling:
   1. 98°C for 30 sec
   2. 98°C for 10 sec
   3. 67°C for 30 sec
   4. 72°C for 45 sec
   5. Go to (b) for 34 additional cycles
   6. 72°C for 2 min
   7. 10°C hold
3. Run reaction on 1% agarose gel.
4. For Sanger sequencing, gel extract/PCR purify. Add 12.5 uL PCR product with 2.5 uL primer (10 uM)
   1. M1 F5 or M1 R6 recommended for Sanger.