Large Scale RNA Extraction Protocol

RNA Isolation:

1. Grind tissues (using liquid N_2) with a mortar and pestle into a powder
2. Add ~2 grams of plant tissue to a 30mL centrifuge tube (Nalgene; 3119-0030)
3. Quickly add 10mL Plant Purification RNA Reagent and vortex ~20 seconds
4. Slowly vortex sample on its side for 5 minutes at a setting of 4 or 5
5. Centrifuge at 16000 rpm for 5 minutes in the Sorvall centrifuge (Rotor: AM 38.15)
6. Pipet supernatant to new 30 mL centrifuge tube and centrifuge at 16000 rpm for 5 minutes.
7. Pipet supernatant to new tube and add 2 mL 5M NaCl. Pipet to mix
8. Add 6mL chloroform and invert to mix
9. Centrifuge at 16000 rpm for 2 minutes
10. Transfer aqueous (top) layer to a new tube
11. Repeat steps 8-10
12. Add an equal amount of isopropanol to the sample and let sit and room temperature for 10 minutes
13. Centrifuge at 16000 rpm for 10 minutes
14. Pour off supernatant and rinse the pellet with 4-5mL 75% EtOH (Possible break point: if necessary, you may leave the pellet/EtOH in -80 ^{o}C overnight and resume procedure the following day.)
15. Air dry pellet for 10 minutes
16. Add 1.78mL 1X RNA Secure Reagent and mix by vortex and pipet
17. Incubate at 65^{o}C for 10 minutes (waterbath in Chang lab)
18. Add 200uL Turbo DNase Buffer and 20uL Turbo DNase (Do Not Vortex!)
19. Digest at 37^{o}C for 10 minutes (Incubator in Chang Lab)

RNA Clean up:

1. 1. Add 7 mL new RLT/RLC buffer and mix well by pipeting
    a) RLT Recipe: For every 1mL RLT or RLC buffer (in Qiagen Kit) add 10uL of mercaptoethanol (BME)
2. Centrifuge at max speed for 2:00 minutes
3. Add 5mL 100% ethanol and mix well. Do not Centrifuge!
4. Transfer 735uL of sample to pink minispin column (use 2 per sample) (in Quiagen Kit) and centrifuge for 30 seconds at 10,000 rpm. Discard flow through and repeat. 2X.
5. Add 500uL RPE or PE buffer and centrifuge for 30 seconds at 10,000 rpm. Discard flow through and repeat, allowing to centrifuge for 2:00 minutes.
6. Discard flow through and centrifuge again for 1:00 minute.
7. Transfer column to new 1.7mL tube , add 32uL nuclease or RNase free water (located on RNA only shelf) directly on the membrane of the column, being careful not to puncture or poke the membrane.
8. Centrifuge for 1:00 minute at 10,000 rpm. Place spin column in a 2mL collection tube
9. Repeat steps 5 – 9 until all of sample is eluted. (usually 3 times total). Store at -80^{o}C

30 mL tube clean-up (Nalgene 3119-0030): Nalgene tubes are reusable.

1. Wash out tubes with Alconox detergent
2. Rinse thoroughly with deionized water (multiple rinses)
3. Rinse out with 95% EtOH
4. Rinse 3 times with double distilled water
5. Spray inside of tubes with RNase inhibitor (Ambion; AM9780)