

## RNA Prep Protocol

### Preparation:

Autoclave tips

Autoclave 1.5ml tubes

Autoclave metal scraper

RNAse away pipettes

RNAse mortar and pestle

### Procedure:

1. Add 3mL of trizol into a snap cap tube (or corex tube) in the hood
  2. Add liquid nitrogen into a mortar and pestle along with the metal scraper
  3. Weigh 0.15g-0.20g tissue in weigh boat (immediately put tissue back)
  4. Add tissue to mortal and grind until the liquid nitrogen has evaporated
  5. Scrape tissue quickly into trizol
  6. Grind tissue with the homogenizer (\*Don't put fingers near the bottom of the snap cap tube)
  7. Grind slower at first to get all the pieces wet
  8. Grind at 8.5 for 45 seconds
  9. Clean the homogenizer with DEPC H<sub>2</sub>O in a flask (you can also remove the grinder piece by lifting up to better clean)
  10. Incubate at RT for 5 minutes (allows for dissociation of RNA from nuclear proteins)
  11. Centrifuge in the ultra speed centrifuge in the snap cap tube along with a sleeve for 10 mins at 6000 rpm at 2-8C
  12. Transfer supernatant into 2 labeled 1.5ml tubes in the hood (1ml in each tube)
  13. Add 0.2ml of chloroform (be careful because it drips very easily out of the tips) and shake vigorously for 15 sec
  14. Incubate at RT for 2-3min
  15. Spin at 12,000g for 15 min at 4C
  16. Transfer top layer into a new 1.5 tube (it's better to leave a little behind than get contamination of the interphase)
  17. Repeat steps 13-16 two more times (3 chloroform extractions total)
- Note: for RNA isolation from kernels, may want additional chloroform extractions
18. Add the following to the transferred supernatant:
    - a. 0.25ml isopropanol
    - b. 0.25ml high salt
    - c. 2µl glycogen
  19. Mix and incubate for 10 min
  20. Spin for 10 min at 12,000g
  21. Pour off supernatant
  22. Add 1ml of 75% EtOH and vortex until the pellet is released from the bottom of the tube
  23. Pour off supernatant
  24. Let the pellet dry for 5-6 mins at RT (pull down window on hood)
  25. Add 400µl DEPC H<sub>2</sub>O and 1µl Rnasin

26. Resuspend pellet by vortexing (heat if needed at 60-65C)
27. Add 200µl 6M LiCl on ice for 2-4 hrs or 4C overnight
28. Spin at 14,000g for 5 min
29. Pour off supernatant
30. Wash pellet with 1 ml 75% EtOH and vortex
31. Spin for 5 min at 7,500 rpm
32. Remove supernatant
33. Air dry for 5-6 min
34. Resuspend in 50µl DEPC H<sub>2</sub>O + 1µl RNAsin
35. DNase treat by adding 1µl of DNase
36. Incubate at room temperature for 15 min
37. Incubate at 65 for 15 min
38. Run 5µl on gel at 160V for 10-15min
  - a. should see 2 bands
  - b. will have some degradation due to gel
  - c. alternatively you can use a formaldehyde gel that does not denature the RNA
39. Spec on the nanodrop in Margi's lab
  - a. Click on nanodrop icon
  - b. Place 1µl of DEPC H<sub>2</sub>O on the nanodrop
  - c. Lower lid all the way down and release up
  - d. Click on blank button
  - e. Wipe nanodrop with a kim wipe very gently to remove DEPC
  - f. Place 1µl of sample on the nanodrop
  - g. Click on measure button
  - h. Good RNA characteristics
    - i. 260/280 ratio can be 1.9-2.1 (ideal is 2-2.1)
    - ii. 260/230 ratio 2-2.4
    - iii. high concentration