RNA Isolation from Tissue Samples

Supplies Needed:

- Polypropylene tubes (#2059 Falcon Tubes) – 1/sample
- Eppendorf microfuge tubes – 2 to 3/sample
- Chloroform – Pour into a 50ml conical polypropylene tube
- Isopropanol – 50ml conical
- 75% Ethanol – Use Dep C water
- RNA-zol solution – Keep on ice
    - 2-3ml in #2059 Falcon tube/sample

Homogenization

- In a fume hood, add 2ml RNA-zol/100mg tissue sample to a #2059 Falcon tube
- Place tissue in a Petri dish containing a small amount of liquid nitrogen. (Tissue must remain frozen at all times.) Using a straight edge razor blade, cute a 100-200 mg piece of tissue into small pieces. Add the frozen tissue to the RNA-zol in the tube.
- Lower the polytron probe into the bottom of the tube and homogenize the tissue sample at least one minute at high speed. Turn off probe and slowly remove the probe. (Do not run the probe dry)
- Place tubes on ice until all samples are homogenized. Wait at least 5 minutes before going to next step
- Between each sample, wash the probe in three beakers of MQ-H_2O. Wipe the probe dry with a clean kimwipe between each beaker and before starting the next sample.

RNA Extraction

- Add 200ul chloroform/2ml RNA-zol to the homogenate. Cap tightly. Vortex for ~15 secs.
    - The homenate must be a milky white or it will not separate properly when centrifuged.
    - Add additional chloroform in 100ul increments until homogenate is milky white.
- Place all tubes on ice for 15-20 mins
- Centrifuge for 15-20 mins at 10,000rpm in Beckman-Coulter Super Speed.
- Carefully remove 700ul of the top, clear, aqueous phase of the fluid and transfer to a fresh eppendorf tube. Do this twice.
- Leave about 2cm of the clear liquid on top of the middle interface layer. Cape and discard the lower organic phase.

RNA Precipitation

- Add an equal volume of isopropanol to the aqueous phase in th eeppendorfs.
- Vortex about 30 secs and store a -20˚C for at least 45 mins (or overnight).

  - There should be a white layer at the bottom of the tube which is the RNA precipitating from the solution
- Centrifuge samples for 15-20 mis at 12,000g at 4˚C.
  - The RNA will form a white pellet at the bottom of the tube
  - Keep pellet in -80˚ overnight if needed

RNA Wash

- Remove the supernatant by pouring off or pipetting.
  - It is best to remove as much liquid as possible especially if stopping at this point.
- Add 600ul 75% EtOH to the pellet in the tube, vortex until the pellet is free floating and centrifuge at 12,000 g for 8 mins.
  - Store at -80˚C before vortexing if needed
- Wash the pellet twice in EtOH
- Remove all supernatant after second wash and place in speed vacuum for 2 mins or until pellet is practically dry.
- Add 20-100 ul Dep C waqter to the pellet and allow to set at room temperature for 15 mins or until the pellet is dissolved
  - If the pellet does not dissolve, the tube may be placed at 37˚ C for 15 mins
- The RNA sample is now ready to quantitate

Notes

- *Wear gloves during the entire procedure to prevent RNAse contamination.*
- *Use sterile tips, tubes, etc*
- *RNA-zol contains irritants and poisons (guanidinium, thiocynate, phenol, and 2-mercaptoethanol)*
  - *wear gloves, goggles, and lab coat*
  - *Work in a fume hood whenever possible.*
  - *Be sure to cap all tubes containing RNA-zol and place in red biohazard bag*
    - *Tie biohazard bag before disposal*
  - *DO NOT GET IN EYES OR ON SKIN OR CLOTHING. AVOID BREATHING IN THE VAPORS*