RNA extraction from tubers

Safety tip: All work is done in fume hood. Use a large shallow tray with a bench diaper as a workspace to protect against dangerous spills. Safety glasses, nitrile gloves, and full-length sleeves MUST be worn. A dry run with baths, mixers, etc. is highly recommended. There is a possible phenol burn hazard and the procedure uses chloroform (toxic!).

1. Mix equal volumes (~50ml each) of Extraction buffer and equilibrated phenol in a clean Pyrex bottle. Preheat the mixture in an 85°C water bath before using.
   Extraction buffer = 100mM LiCl, 100mM Tris pH 8, 10mM EDTA, 1% SDS
   Equilibrated phenol = water saturated, equilibrated with Tris pH 7.9
   Pre-label 2 centrifuge tubes for each sample. Samples should be processed in groups equal to the capacity of one swinging bucket rotor (JA13.1 = 6)

2. For each sample, transfer frozen tuber powder into a labeled, sterile 50 ml Falcon 2098 tube (chloroform resistant) and store in liquid nitrogen until needed. Tubes are filled to approximately the 20 ml mark with frozen powder and kept in liquid N_2 until use. (Frozen tuber powder is normally prepared by grinding with Omni Mixer under liquid nitrogen)

3. Take one tube of frozen powder, loosen cap and place in rack. [Warming powder just a bit before adding liquid helps—too much powder will make this step difficult. The goal is to warm the frozen powder without really thawing it.]

   Pull Extraction buffer/phenol from the bath and carefully mix until there is no interface. Pipette 15 ml of hot extraction buffer/phenol into tube of frozen powder. Tighten cap and vortex vigorously (be careful!)* until all of frozen powder is suspended in liquid. Work quickly and furiously to get it resuspended. Turning tube upside down and banging gingerly on cap-end (which is wrapped in a Kimwipe) may dislodge frozen powder at conical end of tube—safer plan is to vortex the tube on its side. Give one tube your entire attention and it will give better results than trying to double up tubes. The overall procedure (steps 3-6) can take a while and rest on ice with re-mixing, but step #3 must be done as quickly as possible for each individual sample.

   *Periodically during the mixing, the cap should be loosened (and then retightened) to allow pressure to escape. A Kimwipe wrapped around the cap area will protect against chemicals spraying and bubbling up out of tube.

4. Add 7.5 ml chloroform and mix again—just enough to mix ingredients well. Quickly—the tube will melt, if you lag. Here again, if the powder is already mixed completely in the phenol mix, this step should be a single vortex, before transferring it to a resistant tube.

5. Transfer to a chloroform resistant pre-labeled centrifuge tube (Oak Ridge Polypropylene, 30 ml) and set tube in ice bucket while processing other samples. (Sample volume should be around 30 ml.)

6. Repeat steps 3-5 until you have a rotor-full of samples. Work quickly and mix all samples in bucket each time a new sample is added.

7. Centrifuge samples in Beckman JS 13.1 @ 4°C 11,000 rpm for 10 minutes (~19,000 x g)

8. Transfer top layer of liquid (aqueous phase) to a fresh pre-labeled centrifuge tube. Avoid the interface even if it means sacrificing sample! Volume should be approximately 12ml. Add equal volume of 4M LiCl, mix well and incubate @ -20°C for 30 minutes-1hr. After about 45 minutes in the freezer some samples may begin to freeze solid. Nothing is gained or lost by leaving frozen samples in freezer. If samples freeze solid or slushy, thaw to a 4°C liquid and mix before centrifuging.

9. Centrifuge samples in Beckman JS 13.1 @ 4°C 11,000 rpm for 20 minutes. Pour off supernatant and turn tubes upside down to dry. Wipe excess liquid from tube walls before turning upright. Add 500 µl of sterile water to the bottom of the tube. Resuspend pellet (which might be invisible) by repeatedly washing bottom of the tube with the water. Using a fixed angle rotor may increase yield here.

10. Transfer the 500µl of water to a microcentrifuge tube and do phenol, phenol/chloroform, and then chloroform extractions. Avoid the interface even if it means sacrificing sample! Precipitate with ETOH and Na-acetate. Wash pellet with 70% ETOH. Dry briefly in Speed Vac or on bench top. Very dry RNA is hard to re-suspend!! This second round of phenol and chloroform removes tuber gluck from the RNA pellet—avoid the white interface!

11. Dissolve the pellet in 50µl. Scan a 1:100 dilution to quantify RNA. Dilute RNA solutions are a greater risk for degradation. Concentrated RNA solutions are harder to quantify correctly.

See notes on leaf RNA prep for more tips on the final step, gel handling and blotting.