Extraction of RNA from Potato Leaves

Safety tip: This procedure uses hot, corrosive liquids containing phenol and chloroform, which is toxic. Always wear hand, eye and skin protection and work in a fume hood. Ultra cold liquids also present hazards!
Dispose of waste properly!

- Harvest fully expanded, young leaves from plants. Store the explants in Petri dishes containing filter paper dampened with sterile water.
- Unwounded control leaves are frozen immediately by immersion in liquid nitrogen (LN_2).
- To wound samples, crimp the leaf with a hemostat (serrated jaw) in three or four places. The wound site will appear as dark streaks on the surface of the leaf - don't be shy. Store in Petri dishes with wet filter paper during the wound induction period e.g. minutes or hours post-wounding.
- At the end of the induction period, transfer the tissue to LN_2.
- For immediate processing, the wounded tissue is placed in a mortar sitting in LN_2. It is then ground in the frozen state under LN_2 to a fine powder. After grinding, transfer the powder to a cold BlueMax tube (Falcon 2098) and store at -80°C°C or in LN_2 until needed.
- Make a 1:1 mixture of Extraction Buffer and phenol. Preheat to 90°C in a water bath.

  Extraction buffer = 100mM LiCl, 100mM Tris pH 8, 10mM EDTA, 1% SDS
  Equilibrated phenol = water saturated, equilibrated with Tris pH 7.9

  NOTE: Process samples individually and quickly. It is not necessary to use RNase free tubes or glassware until phenol extractions are complete. Speed is one of the most important ingredients here!

- To an individual sample, add 10 ml of hot (90 °C) buffer/phenol. Vortex until the frozen power has thoroughly mixed with the hot liquid.
- Add 5 ml chloroform. Vortex 5 seconds.
- Immediately transfer to a centrifuge tube and spin at 11,000 rpm in a JS 13.1 swinging bucket rotor for 10 minutes at 4°C.
- Carefully transfer the supernatant liquid to a clean tube and add an equal volume of 4M LiCl. Mix well and incubate at -20 °C for 30 to 45 minutes.
- Centrifuge as above.
- Decant the supernatant liquid and suspend the pellet in 500 µl sterile, DEPC-treated deionized water. Transfer to a microcentrifuge tube and spin for 5 minutes at top speed. Transfer the supernatant to a new tube.
- Perform phenol, phenol/chloroform, and chloroform extractions using equal volumes of reagent and sample.
- Add 50 µl 3M sodium acetate and 1 ml 95% ethanol; mix well.
- Centrifuge 5 to 10 minutes at 4°C. Decant the supernatant and dry the pellet in a vacuum. Suspend the pellet in 50 µl sterile, DEPC-treated deionized water.
- Quantify a 1:100 dilution by absorbance at 260 nM.
- Use 20 µg of RNA per lane for Northern blot analysis.