ANOTHER ARABIDOPSIS RNA EXTRACTION

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* We have isolated RNA from a range of plant species (e.g., lettuce, pea, Pharbitis and wheat) and types of tissue (leaves, cotyledons, seeds and floral tissue). These methods have now been used to isolate total RNA and mRNA from Arabidopsis thaliana. They have proved satisfactory for isolating undegraded RNA that has been used to construct cDNA libraries, carry out in vitro translation and analyse RNA transcripts by Northern blotting. Yields for both of these methods are 200-300 ug-1 fresh weight of Arabidopsis leaf tissue, depending upon the age and growth conditions of the plants.

* With all procedures for the isolation of intact RNA it is extremely important to ensure that equipment and reagents are RNAase free. We routinely use diethyl pyrocarbonate (DEPC) treated water, baked glassware and sterile polypropylene tubes. Molecular biological grade reagents are used when available or alternatives of the highest quality.

1. SOLUTIONS
   (i) EXTRACTION BUFFER
   50mM Tris-HCl (pH 8.0)
   4% (w/v) p-Aminosalicylate
   1% (w/v) tri-isopropyl-naphthalene sulphonate, sodium salt (Phase Separations Ltd)
   2% (v/v) b-Mercaptoethanol.

   (ii) PHENOL REAGENT
   50mM Tris-HCl (pH 8.0) saturated phenol (containing 0.1% [w/v] 8-hydroxyquinoline):chloroform:iso-amyl alcohol (25:24:1 [v/v/v]).
   * Glass distilled aqueous phenol is supplied by Rathburn Chemicals Ltd (Walkerburn, Scotland).
   * Due to the caustic nature of phenolic compounds care should be taken in the use of this reagent and extractions should be in a fume cupboard.

   (iii) LiCl
   12.0M LiCl (filter sterile)

   (iv) CsCl BUFFERS
   40% CsCl in 10mM Tris-HCl (pH 7.5) 5.7M CsCl
   1.0mM EDTA 0.1M EDTA (pH 7.5)
   * The solution is treated with DEPC and autoclaved after 30 min (Take care with DEPC).

2. SMALL SCALE PREPARATION
   (i) Grind 1 g fresh weight of leaf tissue to a fine powder in liquid nitrogen using a mortar and pestle.
   (ii) Transfer the powder to sterile Corex tubes (Dupont), containing an 10 ml each of phenol reagent and extraction buffer.
   (iii) Blend the mixture at three quarter speed for 2 min using the small probe (S25 N10G) attachment for an ultra-turrax (Sartorius).
   (iv) Centrifuge in a Sorvall SS34 rotor for 20 min at 7,000rpm (5,864xg).
   (v) Carefully remove the upper aqueous phase to a sterile
polypropylene tube. Re-extract the lower phase with an equal volume of extraction buffer and pool the aqueous phases.

(vi) Precipitate the nucleic acids by addition of 0.1 vol of 3M sodium acetate (pH5.6) and 2.2 vol of ethanol -20oC.
(vii) Mix gently and leave for >2 h at -20oC.
(viii) Collect the nucleic acid precipitate by centrifugation in a Sorvall SS34 rotor at 13,000 rpm (20,190xg) for 30 min.
(ix) Discard the supernatant. Briefly dry the pellet before resuspending it in (usually 500ul ) water for LiCl or in 40% CsCl buffer for CsCl method (see below)
* Any undissolved material will go into solution on addition of more water. However, it is important not to dilute the nucleic acid concentration substantially as the efficiency of subsequent LiCl precipitations can be reduced.

(x) Transfer the nucleic acid solution into 1.5 ml Eppendorf tubes.
* RNA can now be selectively isolated using either LiCl or CsCl.

3. LiCl ISOLATION OF RNA
* LiCl selectively precipitates large mw ribosomal and mRNA from DNA and small mw tRNA.

(i) Add LiCl to the total nucleic acid solution to a final concentration of >2M.
(ii) Leave o/n on ice and then collect the precipitated RNA by centrifugation in a microfuge.
(iii) Wash the precipitate with 3.0M sodium acetate (pH 5.6). To remove any small amounts of contaminating DNA.
(iv) After centrifugation, discard the supernatant. Remove the sodium acetate by washing in 70% ethanol (-20oC).
(v) Dry the RNA under vacuum for 1.5 min and resuspend in sterile water.
(vi) Scan the RNA absorbance from 320 nm to 220 nm.
* An absorbance at 260 nm of 1.0 is equivalent to 40 ug ml-l and the 260:280 ratio should be approximately 2.0 for pure RNA. Absorbance at 230 nm is indicative of carbohydrate/ polyphenol contamination.

4. CsCl ISOLATION OF RNA
* The main advantage of this method is that a number of samples can conveniently be prepared together.

(i) Resuspend the total nucleic acid precipitate in <3 ml of 40% CsCl buffer.
(ii) Place 1 ml of this solution into a 4.2 ml polyallomer tube and underlay with 1 ml of 5.7M CsCl.
(iii) Fill the tubes with the rest of the nucleic acid solution to within 2 mm of the top of the tube.
(iv) Centrifuge in a Beckmann SW 60Ti rotor at 31,000 rpm (100,000xg) o/n.
(v) Remove the supernatant. Resuspend the precipitate in 300 ul of 10mM Tris-HCl (pH 7.5), 5mM EDTA and 0.1% (w/v) SDS.
(vi) Transfer the solution to an Eppendorf tube and spin briefly.
(vii) Reprecipitate the RNA by the addition of 3M sodium acetate (pH5.6) and 2.2 vol of ethanol.
(viii) Wash the pellet in 70% ethanol. Dry, resuspended and scan as above.