3.2.3. Selection of Poly(A)$^+$ mRNA

If the proportion of viral RNA is very low compared to plant total RNA, it may be helpful to select for viral and plant mRNAs using an oligo(dT)-cellulose column. This is a modification of the method of Guilford and Forster ***(14)***, and is performed as follows:

1. Add oligo(dT)-cellulose to loading buffer A to make a final concentration of 0.5 mg/mL. Pour 1 mL of this solution into a sterile siliconized Pasteur pipet plugged with siliconized glass wool (*see* **Note 11**).
2. Wash the column in 3 vol each of DEPC-dH_2O and solution A. Test the effluent to ensure that the pH of the column is maintained between 6.0 and 8.0. Wash column with 5 vol of loading buffer A.
3. Heat RNA sample at 65°C for 5 min and add an equal volume of loading buffer A. Cool the mixture to room temperature and apply to the column.
4. Wash the column in 4–6 vol of loading buffer A, then in another 4–6 vol of loading buffer B.
5. Elute poly(A)$^+$ RNA with 3 vol of solution B. Add 3*M* sodium acetate, pH 5.2 (final concentration of 0.3*M*), to elute RNA. Add 2.5 vol 95% ethanol to precipitate RNA, and store at –20°C.
6. Pellet the RNA by centrifugation at 5800*g* at 4°C and resuspend in TE buffer. Determine RNA concentration by UV absorption.

3.2.4. Isolation of Polyribosomal RNAs

Translationally active RNAs can be isolated by extracting polysomes and purifying them on a sucrose cushion. The ribosomes remain tightly bound to the mRNA by incubation with puromycin, and polysomes can be isolated by centrifugation on a sucrose gradient. Polysomal RNAs were isolated by the method of Palukaitis ***(28)***.

1. Freeze 2 g of leaf tissue in liquid nitrogen and grind to a powder in a prechilled mortar. Resuspend powder in 3 mL extraction buffer B (*see* **Note 10**).
2. Remove large debris by centrifugation at 12,000*g* for 24 min at 4°C and add Triton X-100 to the supernatant to a final concentration of 1%. Pellet polyribosomes on a 4-mL sucrose cushion by centrifugation at 100,000*g* for 90 min at 4°C.
3. Remove sucrose solution with an aspirator, wash the pellet with 1 mL of water, and store at –20°C until use.
4. Resuspend pellets in 0.2 mL of dH_2O and 0.25 mL of buffer A. Incubate mixture on ice for 15 min, then at 37°C for 10 min.
5. Layer mixture onto a 5–20% (w/v) sucrose gradient in buffer B and centrifuge at 4°C for 4 h at 100,000*g* (*see* **Note 12**).
6. Collect fractions (0.5 mL) on a density gradient fractionator and determine the RNA concentration of each sample by UV absorption. The first peak (top of the gradient) identified contains mostly tRNA, the middle peak(s) contains mRNA